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Dietary conjugated linoleic acid effects on quantitative and qualitative characteristics of pork

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Dietary conjugated linoleic acid effects on quantitative and qualitative characteristics of pork

by

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GENERAL INTRODUCTION

Trends in United States food consumption over the last 70 years indicate an increase of total fat. This increase is due to greater consumption of vegetable fats, especially salad and cooking oils, while consumption of animal fats has decreased. Red meat (beef, lamb, and pork) has been most affected by this trend because of the relatively large amount of saturated fatty acids in their tissues and its proposed role in producing atherosclerosis. Pork and poultry contain 50% or more saturated fatty acids while less than 50% of the fatty acids in beef and lamb are unsaturated (Romans et al., 1985). In addition to species variations, dietary fat can have profound effects on the fatty acid composition of adipose tissue and muscle. Differences in digestive tract physiology are responsible for differences in ease by which nonruminant species deposit dietary unsaturated fatty acids compared to that of ruminant animals (Rule et al., 1995).

In an attempt to locate heterocyclic amines (carcinogens) in cooked ground beef, a mutagenesis modulator (effective inhibitor of uninduced liver S-9) was found (Pariza et al., 1983). This mutagenesis modulator was identified as a series of conjugated dienoic isomers of linoleic acid (octadecadienoic acid) by Ha et al. (1987) and are referred to as conjugated linoleic acid (CLA) from this point forward. Dietary sources of CLA include milk fat, meat products, and vegetable oils. Chin et al. (1992) found CLA in a wide variety of foodstuffs with meat and milk from ruminants having the highest content. Kepler and Tove (1967) discovered that the first intermediate in the biohydrogenation of linoleic acid by the rumen bacteria, *Butyrivibiro fibrisolvens* is c-9, t-11-18:2, which suggests that ruminants can convert linoleic acid to CLA explaining CLA's greater concentration in ruminants.

The fact that the concentration of CLA differs between animal products and the early reports that CLA is a mutagenesis modulator suggests that CLA could enhance both the actual and perceived healthfulness of meat products. The role of CLA as a potential anticarcinogen has been studied in many different tissues including skin (Ha et al., 1987, and Belury et al., 1996), mammary (Ip et al., 1991, Ip et al., 1994, and Ip and Scimeca, 1997), colon and prostate (Liew et al., 1995, and Cesano et al., 1998), and lung (Schønberg and Krokan, 1995). Atherosclerosis has been studied in rabbits (Lee et al., 1994) and hamsters (Nicolosi et al., 1997) demonstrating that CLA inhibits aortic plaque formations in these species.

Immune function/response has been evaluated in several models and CLA has been found to stimulate macrophages to produce cytokines (Cook et al., 1993). CLA has also been shown to affect eicosanoid regulation of cytokine production (Belury and Kempa-Steczko, 1997, and Turek et al., 1998). Another physiological effect of cytokines is the stimulation of bone resorption leading to weakening of the bones. Since CLA modulates cytokine production, it has been shown to positively affect bone growth (Watkins et al., 1996, Watkins et al., 1997, and Li and Watkins, 1998). Early studies suggested that CLA had antioxidant activity both *in vivo and in vitro* (Ha et al., 1990, and Ip et al., 1991). Van den Berg et al. (1995), Chen et al. (1997), and Banni et al. (1998) have disputed this hypothesis in recent studies.

The concentration of CLA in milk and milk products is related to lactation number (Lal and Narayanan, 1984), diet (Jiang et al., 1996, Stanton et al., 1997, and Kelly et al., 1998), and processing conditions (Shantha et al., 1992, Werner et al., 1992, and Lin et al., 1998). The concentration of CLA in meat was evaluated by Chin et al. (1992) and Shantha et

al. (1994). CLA has been shown to have positive effects on feed conversion and fat deposition in rats, mice, and chickens (Chin et al., 1994, and Park et al., 1997). Similar results have been observed in pigs by Dugan et al. (1997), Dunshae et al. (1998), and Cook et al. (1999).

This project was developed to investigate the overall effects of feeding CLA on pigs. Several areas of study were undertaken. The first was to determine the effects of varying dietary amounts of CLA on performance, body composition and fatty acid deposition. The second was to evaluate the effects of CLA on sensory and quality characteristics of fresh pork loin chops and fresh pork patties. A third study examined the effect of CLA on immune function and blood chemistry parameters of growing-finishing pigs.

Dissertation Organization

This dissertation is in an alternate style format consisting of an abstract, a general introduction, a general review of literature, three papers prepared for publication, and a concluding summary. The three papers represent the work done by the first author to fulfill requirements for the degree of Doctor of Philosophy. The three papers were prepared according to the Journal of Animal Science Style and Form guide. These papers consist of an Abstract, Introduction, Materials and Methods, Results and Discussion, Implications and Literature Cited sections.

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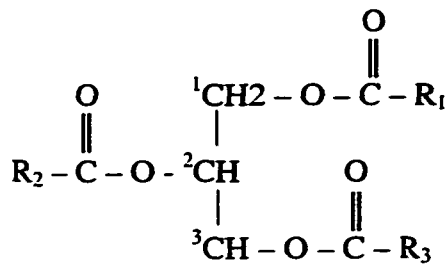
GENERAL REVIEW OF LITERATURE

Lipid Composition

Fats provide a reservoir of energy for animals as fat is the most concentrated form of energy available (Dugan, 1978). Fats consist predominantly of glyceryl esters of fatty acids. Since the glyceride structure is common to all fats, variations in properties from one type of fat to another are due to differences in their fatty acid composition. Fatty acids are long-chain hydrocarbons with a carboxyl group at one end and a methyl group on the other end. They are unbranched and usually have an even number of carbon atoms. Fatty acids with carbon lengths of 16 and 18 predominate in meat, whereas in some species of fish, fatty acids with carbon lengths of 18 to 22 are most common.

The carbon chains of fatty acids which contain only single bonds between adjacent carbons are fully saturated with hydrogen atoms and are thus known as saturated fatty acids (e.g. palmitic and stearic acids). If one or more double bonds appear between adjacent carbon atoms, they are called unsaturated fatty acids (e.g. oleic, linoleic, and linolenic acids). Fatty acids with one double bond are known as monounsaturated and those with two or more double bonds are known as polyunsaturated fatty acids. Unsaturated fatty acids typically have higher iodine values and lower melting points than saturated fatty acids. However, melting point is affected not only by the level of saturation but also by isomeric form and molecular configuration.

In foods the fatty acid moieties are combined by ester linkage to a three-carbon alcohol called glycerol to form triacylglycerols or triglycerides. This is the general structure of a triglyceride:

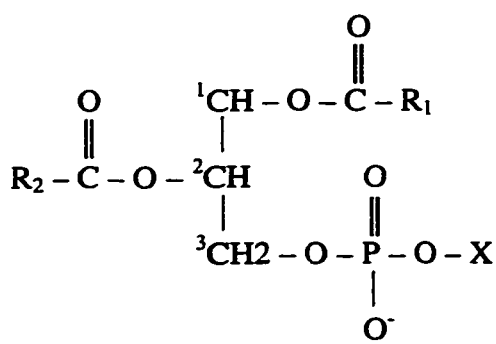


R_1, R_2, and R_3 represent fatty acids

Glycerol may be combined with one, two, or three fatty acids to form mono-, di-, or triglycerides, respectively. Triglycerides are the predominant form of lipids associated with food fats and make up over 98% of all fats in meat.

Saturated and monounsaturated fatty acids predominate in meat fats (Dugan, 1978). Animal fats are typically rich in stearic, palmitic and oleic acids (Dugan, 1978). Pork and poultry contain 50 percent or more unsaturated fatty acids, mainly a result of the dietary linoleic acid from corn (Romans et al., 1983). Beef and lamb contain less than 50% unsaturated fatty acids because the rumen metabolizes dietary fatty acids into saturated fatty acids for deposition in the body (Romans et al., 1983).

Another lipid of interest in meats is phospholipids. Phospholipids contain two fatty acids combined with two of the alcohol groups of the glycerol molecule. The third alcohol group is combined with a phosphate group, which is then combined with another alcohol. This is the general structure for phospholipid:



R_1 and R_2 represent fatty acids, and X may be ethanolamine, choline, serine, inositol, glycerol or phosphatidylglycerol

Phospholipids make up a small concentration (0.5 – 1.0%) of total muscle tissue (Dugan, 1978). Phosphoglycerides are the most common class of phospholipids found in muscle. They are formed when diacyl esters of glycerol bind to a phosphate containing group. The phosphate group is most commonly esterified with choline, serine and ethanolamine (Dugan, 1978). The fact that higher proportions of polyunsaturated fatty acids are incorporated into phospholipids as opposed triglycerides reflects the functional differences of these lipids. Triglycerides are storage depots of lipids in the adipose tissue of animals, however the phospholipids, which are structural components of cell membranes, must remain fluid to allow passage of molecules through them.

It is known that the fatty acid composition and the degree of saturation in pork subcutaneous adipose tissue are affected by a number of factors such as; sex, breed, age, diet, and anatomical location (Jeremiah, 1982). Diet and sex have also been shown to influence fatty acid composition of intramuscular lipids (Malmfors et al., 1978, Girard et al., 1983, and Marchello et al., 1983).

Dietary Lipids on Pork Composition

In pigs and poultry, dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids (Wood and Enser, 1997). The polyunsaturated fatty acids linoleic and α - linolenic cannot be synthesized and tissue concentrations respond rapidly to dietary changes. Saturated and monounsaturated fatty acids however are synthesized and their concentrations are not as easily influenced by diet.

In swine, consumption of high-fat diets also results in marked changes in fatty acid composition. Koch et al. (1968) fed 10% safflower oil and Skelley et al. (1975) fed 30%

roasted, full fat soybeans. Their results of pork subcutaneous adipose tissue for controls ranged from 25-26% 16:0, 9-13% 18:0, 44-46% 18:1, and 11-13% 18:2, and high linoleate-fed pigs ranged from 19-23% 16:0, 8-11% 18:0, 30-37% 18:1, and 21-38% 18:2. These studies indicated that the proportions of 18:2 (linoleic acid) and 18:3 (linolenic acid) increased compared to 18:1 (oleic acid) which decreased. Koch et al. (1968) observed the same response to dietary consumption of safflower oil for inner and outer layers of backfat, perirenal and intramuscular adipose tissue.

A recent addition to sources of high-fat feeds and feed oils is canola (high in 18:1) and flaxseed (high in 18:3). Because of the positive effects that 18:1 has shown in lowering low density lipoprotein cholesterol in humans, incorporation of more 18:1 in lipids of meat animals has been investigated (Rule et al., 1995). The effect of supplemental canola oil (10%) on fatty acid composition of adipose tissue and muscle lipids was studied by St John et al. (1987), Miller et al. (1990), and Busboom et al. (1991). Fatty acid composition in adipose tissue of control pigs ranged from 24-26% 16:0, 12-14% 18:0, 43-47% 18:1, 11-12% 18:2, and 1-2% 18:3, and that of canola fed pigs ranged from 15-22% 16:0, 7-11% 18:0, 46-52% 18:1, 13-18% 18:2, and 2-6% 18:3. Fatty acid compositon in muscle of control pigs ranged from 25-32% 16:0, 9-12% 18:0, 47-49% 18:1, 6-7% 18:2, and 1-2% 18:3, and that of canola fed pigs ranged from 21-24% 16:0, 6-10% 18:0, 46-52% 18:1, 9-14% 18:2, and 1-3% 18:3. In subcutaneous adipose tissue, decreases in saturated fatty acids occurred along with marginal increases in 18:1 and 18:2. The largest increase was in the proportion of 18:3. Nevertheless, the ratio of unsaturated to saturated fatty acids was increased. Although few changes occurred in swine muscle lipids, 16:0 decreased and 18:2 increased somewhat in muscle lipids by feeding canola oil. Busboom et al. (1991) did not observe as large an effect

on intramuscular tissue fatty acids as did Koch et al. (1968). This may be due to the fact that Busboom et al. (1991) fed ground, full-fat canola. Romans et al. (1995) demonstrated that pigs fed 15% flaxseed (high in 18:3) had a two- to sixfold increase in 18:3 and 20:4 (arachidonic acid) in subcutaneous and perirenal adipose tissue and a fourfold increase in 18:3 in longissimus muscle compared to controls.

In general, depot fat of pigs reflect the major fatty acids of the oils they have been fed. For this reason, feeding conjugated linoleic acid (18:2 with conjugated double bonds) was of interest based on its proposed biological activity.

Structure of Conjugated Linoleic Acid

Conjugated linoleic acid (CLA) is a group of positional and geometric isomers of linoleic acid (c-9, c-12-octadecadienoic acid). CLA consists of all combinations of 9,11 and 10,12 positional isomers and c/c, c/t, t/c, or t/t (c = cis, t =trans) geometric isomers. Synthesis of CLA requires the presence of free linoleic acid, a free-radical generating species, and proteins rich in sulfur residues (Dormandy and Wickens, 1987). CLA is currently synthesized for commercial use from sunflower oil. Total conjugated linoleic acid concentration of commercially available CLA from sunflower oil ranges from 58-62%. Approximately 44% of the conjugated dienes of commercially available CLA consists of *cis*9, *trans*11- and *trans*9, *cis*11-octadecadienoate; and *trans*10, *cis*12-octadecadienoate isomers (Cook et al., 1999).

CLA was first identified as a potential anticarcinogen from extracts of grilled ground beef by Pariza et al. (1983). Since cooking many protein-rich foods including ground beef, produces heterocyclic amines which are mutagenic / carcinogenic, the discovery of CLA as a

potential anticarcinogen could enhance both the actual and perceived healthfulness of meat products. Because research in any one area related to CLA is limited, a discussion of each specific research area will follow.

Biological Activity of Conjugated Linoleic Acid

Carcinogenesis

Skin. Initially Pariza et al. (1983) identified a mutagenesis modulator in fried ground beef that was not destroyed by cooking. This mutagenesis modulator was identified as not toxic for bacteria under the conditions of the test, and appeared to act on rat liver S-9 preparation added to the system to activate mutagens for mutagensis. Pariza and Hargraves (1985) suggested that these substances may mitigate the harmful effects of low levels of mutagens and carcinogens ingested with daily food consumption. This work was continued by Ha et al., (1987) which determined that substances in fried ground beef inhibited carcinogen-induced neoplasia in mouse epidermis. Mice treated at 7 days, 3 days, and 5 minutes prior to DMBA carcinogen application at doses of 20, 20, and 10 respectively, developed only half as many pappilomas and had a lower tumor incidence compared to control mice. These substances in fried ground beef were identified as a series of conjugated dienoic isomers of linoleic acid. The four major isomers identified by Ha were (c-9, t-11-; t-9, t-11-; t-10, c-12-; and t-10, t-12-) which accounted for more than 90% of the total. From this point on the mixture was referred to as CLA.

An additional study by Ha and Pariza (1990) showed that CLA inhibited the initiation of mouse forestomach carcinogenesis by benzo(a)pyrene. In this study, a dose of 0.1 ml of CLA was administered by gavage at 4 and 2 days prior to benzo(a)pyrene treatment . After

administration CLA was incorporated into the phospholipid fraction of cell membranes. Nine CLA isomers were introduced, of these nine only the c-9, t-11- was determined to be biologically active because it was the only one found in the phospholipid fraction. The authors suggested that the anticarcinogenic properties of CLA was due to it acting as an *in situ* defense mechanism against membrane attack by free radicals. Belury et al. (1996) investigated the role of increasing levels of dietary CLA in mouse skin tumor promotion by 12-0-tetradecanoylphorbol-13-acetate (TPA). Diets containing 1.0% and 1.5% CLA inhibited tumor yield compared with diets without CLA or 0.5% CLA. A reduction in papilloma incidence was observed in mice fed 1.5% CLA compared with diets containing 0.0 - 1.0% CLA.

Mammary. The chemoprotective effect of CLA on mammary carcinogenesis reported by Ip et al. (1991) was of particular interest because it was the first to show that chemoprotection is an effect of dietary CLA. In this study, as little as 0.5% of CLA was sufficient to cause a significant reduction in the total number of tumors in female Sprague-Dawley rats using the DMBA-induced mammary tumor model. CLA was shown to be cytotoxic to human MCF-7 breast cancer cells by Shultz et al. (1992a) and Shultz et al. (1992b). Their results suggest that tumor growth inhibition may be due to availability of CLA to inhibit protein and nucleotide biosynthesis.

In 1994, Ip et al., determined that the proliferative activity of lobuloalveolar units (sites of carcinogenic transformation) was reduced by feeding CLA and was similar to the bioassay results of tumor inhibition. Exposure to CLA during mammary gland morphogenesis during adolescence (in the rat) may provide lasting protection against subsequent cancer risk. In a group of three studies, Ip et al. (1995) set out to determine how

the timing and feeding of CLA might affect the development of mammary carcinogenesis in the methylnitrosourea (MNU) model. They determined that 1.0% CLA during early postweaning and puberty was only sufficient to reduce subsequent tumorigenesis induced by a single MNU dose. Maximal inhibition of tumorigenesis however required continuous CLA intake after MNU administration, suggesting that some active metabolite (s) of CLA might be involved in suppressing the process of neoplastic promotion / progression. Also in 1995, DesBordes and Lea studied the influence of geometrical isomerism on the growth regulatory effects of 18 carbon unsaturated fatty acids in rat hepatoma and human breast cancer cells. Their results indicate that linoleic acid was more inhibitory than its trans, trans isomer and was also more inhibitory than CLA. These authors suggest that caution is necessary before concluding that CLA has notable growth inhibitory action against cancer cells.

Because conjugated linoleic acid is a naturally occurring fatty acid, consumption of high levels of CLA may also increase total fat intake. Information as to whether an increase in the level of fat or a substitution of the type of fat in the diet may effect the cancer inhibitory efficacy of CLA had not been investigated. Ip et al. (1996) studied whether the anticarcinogenic activity of CLA is affected by the amount and composition of dietary fat consumed by the rat. The results indicate that the magnitude of tumor inhibition by 1% CLA was not influenced by the fat level (10, 13.3, 16.7, or 20% by weight in the diet) or type (corn oil or lard) in the diet. In addition, the authors concluded that the cancer preventive activity of CLA is unlikely to be mediated by the interference with the metabolic cascade involved in converting linoleic acid to eicosanoids. Another study by Ip et al. (1997) evaluated how changes in the concentration of CLA in mammary tissues as a function of CLA exposure / withdrawal were correlated with the rate of occurrence of mammary carcinomas in rats.

Significant tumor inhibition was observed only in rats that were given CLA for the entire duration of the experiment (20 weeks). It was also noted that incorporation of CLA was much higher in neutral lipids than in phospholipids of the mammary gland. Removal of CLA from the diet returned basal values of neutral lipid- and phospholipid-CLA in about 4 and 8 weeks, respectively. The rate of disappearance of neutral lipid CLA subsequent to CLA withdrawal paralleled more closely the rate of occurrence of new tumors. In subsequent study, Ip and Scimeca (1997) evaluated whether there was an interaction between linolec acid and conjugated linoleic acid. Analysis of neutral lipids and phospholipids of the mammary tissue indicated that 1) the accumulation of CLA in mammary tissue was dose dependent from 0.5% to 2%, 2) CLA concentration was ten times higher in neutral lipids than in phospholipids, 3) the incorporation of CLA in either fraction was not affected by the availability of linoleic acid, and 4) CLA did not appear to displace linoleic acid or arachidonic acid in the mammary tissue.

Two studies involving the effects of CLA on MCF-7 human breast cancer cells were reported by (Durgam and Fernandes, and Cunningham et al.) in 1997. Durgam and Fernandes examined whether the inhibitor action of CLA (control, 1.7×10^{-5}, 3.5×10^{-5}, and 7.1×10^{-5} incubated for 6 days) is related to estrogen responsiveness. Their results demonstrate that CLA negatively regulates hormone mediated breast cancer cell growth. Cunningham et al. (1997) compared potential mechanisms for stimulation or inhibition of cell growth by linoleic acid (1 μg / mL) and CLA (1 μg / mL) by using eicosanoid synthesis inhibitors. The addition of cyclooxygenase to CLA supplemented normal human mammary epithelial cells and MCF-7 breast cancer cells stimulated the growth of normal and cancer cells. However, the addition of lipoxygenase to CLA supplemented MCF-7 breast cancer

cells resulted in synergistic growth inhibition, suggesting that the growth suppression was augmented by CLA through inhibition of leukotriene synthesis.

Wong et al. (1997) studied the effects of CLA on established murine mammary tumors. They found that CLA had no obvious effect on the growth of an established, aggressive mammary tumor. Visonneau et al. (1997) studied the growth and mestatic spread of transplantable mammary tumors. Their results indicated that 1.0% dietary CLA administered 2 weeks prior to inocculation blocked both the local growth and systemic spread of human breast cancer to lungs, peripheral blood, and bone marrow.

Colon and Prostate. The effect of CLA against 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) induced colon carcinogenesis was studied by Liew et al. (1995). This investigation determined that during the initiation phase of carcinogenesis, CLA protects against IQ-DNA adducts and colonic aberrant crypt foci in the colon of the F344 rat. Cesano et al. (1998) studied the effect of three different diets (standard, 1.0% linoleic acid, or 1.0% CLA added) on the local growth and mestatic properties of DU-145 human prostatic carcinoma cells in SCID mice. Mice receiving linoleic acid supplemented diet displayed increased local tumor loads than compared to the other two groups. Mice fed the CLA-supplemented diet displayed smaller local tumors and a major reduction in lung metastasis.

Lung. Schønberg and Krokan (1995) examined the effects of linoleic acid and conjugated linoleic acid on three different lung adenocarcinoma cell lines (A-427, SK-LU-1, A549) and one human glioblastoma cell line (A-172). CLA (control, 10 μM, 20 μM, and 40 μM) exhibited a dose dependent reduction in proliferation of the lung adenocarcinoma cell lines. A-427 was the most sensitive and A-172 was virtually not effected. Linoleic acid had no inhibitory effect on either cell line.

Atherosclerosis

Atherosclerosis was evaluated in rabbits fed .5g CLA/rabbit per day by Lee et al. (1994). They found that by 12 weeks, total and LDL cholesterol and triglycerides were lower in the CLA-fed group. The LDL cholesterol to HDL cholesterol ratio and total cholesterol to HDL cholesterol ratio were significantly reduced in CLA-fed rabbits. When aortas were examined the CLA-fed rabbits showed less atherosclerosis.

Hypercholesterolemic hamsters were fed CLA (control, .06%, .11% and 1.1%) in a study by Nicolosi et al. (1997). They also reported that CLA reduced the fatty streak formation in arteries and reduced total serum cholesterol levels. Linoleic acid (1.1%) was more effective at reducing total serum cholesterol and triglyceride levels, however CLA was five times as effective in decreasing plaques on arterial walls. These results suggest that serum lipid changes alone may not be an accurate measure of the antiatherogenic effects of CLA.

Immune Function

The effects of CLA on fat deposition and growth may be due to its modulation of various parts of the immune system. Normally, factors that stimulate the immune system, such as vaccinations or vitamin C (Chamblee et al., 1992), stimulate macrophages to produce cytokines and this, causes catabolism of muscle cells (Roura et al., 1991).

It has been suggested that maximal immune response is also the optimal (Cook et al., 1993), however studies designed to test this theory suggest otherwise. The performance of growing chicks was adversely affected by immune stimulation in studies by Klasing and Austic (1984) and Klasing et al. (1987). Their studies found that reduced growth and feed intake resulted from an injection of sheep red blood cells (SRBC) or an endotoxin

(lipopolysaccharide, LPS). These decreases in growth and feed intake was also noted when interleukin-1(IL-1), a cytokine produced by macrophages following immune stimulation was injected by Klasing et al. (1987). Furthermore, IL-1 injection enhanced skeletal muscle degradation and depressed skeletal muscle synthesis.

Cook et al., (1993) fed 0.5% CLA to chicks and rats to determine the ability of CLA to prevent reduced growth rate following endotoxin (lipopolysaccharide, LPS) injection. Their study found that chicks fed CLA and injected with LPS continued to grow, while those not fed CLA either failed to grow or lost weight following LPS injection. In rats, both controls and those fed CLA lost weight initially after LPS injection, however CLA fed rats lost only half the body weight of the controls. The authors concluded that dietary CLA had no adverse effects on immune variables and did prevent the catabolic effects of immune stimulation in both chicks and rats.

To determine the ability of CLA to prevent endotoxin-induced growth suppression, Miller et al. (1994) fed mice diets containing fish oil, CLA, or a basal diet. Conjugated linoleic acid prevented anorexia caused by endotoxin injection. Splenocyte blastogenesis was increased by CLA. These results supported those by Michel et al. (1992) which reported that CLA stimulated mitogen-induced lymphocyte blastogenesis, cytotoxic activity and macrophage killing ability. Belury and Kempa-Steczko (1997) determined that CLA is a suitable substrate for Δ6 desaturase, membrane-bound CLA. Desaturated and / or elongated products of CLA are likely to compete with other PUFA for phospholipase, cyclooxygenase, and lipoxygenase enzyme families. They also suggest that dietary CLA modulation of Δ6 desaturase activity may reduce phospholipid arachidonate, resulting in an overall decrease in arachidonate-derived eicosanoids. Arachidonic acid is the precursor of a group of hormones

known as protaglandins. A decrease in prostaglandin production may affect such biological functions as; lypolysis, fluid balance, gastrointestinal function, and estrus.

In 1997, Chew et al. hypothesized that the potential anticancer activity of CLA may be mediated through enhanced immune activity. To test this hypothesis they studied CLA (control, 1.78×10^{-5}, 3.57×10^{-5}, and 7.14×10^{-5}) and β-carotene (control, 10^{-9}M, 10^{-8}M, 10^{-7}M) both independently and together on *in vitro* porcine blood lymphocytes and murine peritoneal macrophages. Their results suggest that CLA stimulated lymphocyte proliferation when cells were stimulated with both T-cell antigens pokeweed mitogen (PWM), and concanavalin A (con A) and with the T-cell dependant B-cell mitogen (PHA, phytohemagglutinin). CLA also stimulated cytotoxic activity of lymphocytes and the bactericidal activity of peritoneal macrophages. In contrast to these results, CLA inhibited interlukin-2 (IL-2) production by lymphocytes and suppressed the phagocytic activity of macrophages. When CLA and β-carotene were present together, they interacted additively to further enhance both lymphocyte cytotoxicity and spontaneous lymphocyte proliferation. β-carotene was also able to negate CLA's inhibitory effect on phagocyte activity of macrophages. The authors conclude that CLA and β-carotene enhance the immune system and they may act in concert to further modulate immunity.

Also in 1997, Sugano et al. measured lymphatic recovery, tissue distribution, and metabolic effects of CLA (1.0%)in male Sprague-Dawley rats. Apparent lymphatic recovery of CLA was much lower than for linoleic acid. Not all CLA constituents were equally recovered, and more trans,trans- isomers were recovered than cis,trans or trans,cis-isomers in relation to the composition of CLA given. No effects were observed on serum and liver lipid levels, but the concentration of PGE_2 in serum and spleen tended to be reduced by CLA.

Conjugated linoleic acid did not increase tissue thiobarbituric acid (TBA) values. In 1998, Sugano et al. investigated the effects of CLA on the levels of chemical mediators in peritoneal exudate cells, spleen and lung, and the concentration of immunoglobulins in mesenteric lymph node and splenic lymphocytes and in serum of rats. There was a trend toward a reduction in the release of leukotriene B_4 (LTB_4) from the exudate cells in response to dietary CLA level. CLA did not affect the release of histamine. Differences between the control and 1.0% CLA groups were significant for splenic LTB_4, lung LTC_4 and PGE_2 levels with CLA controlling the production of these factors. Splenic levels of immunoglobulin A (IgA), IgG, and IgM increased while IgE decreased in animals fed 1.0% CLA. This was also found in serum levels of immunoglobulins. No differences were seen in the proportion of T-lymphocyte subsets of mesenteric lymph node.

To determine if CLA altered cytokine and eicosanoid production in a manner similar to (n-3) PUFA, Turek et al. (1998) conducted a study with Sprague-Dawley rats fed for 42 days. Diets included soybean oil, and menhaden + safflower oil, with or without CLA at 10g / kg. The extent of CLA incorporation into the spleen was determined by fatty acid analysis, and peritoneal macrophage tumor necrosis factor (TNF), interlukin-1 (IL-1), and interlukin-6(IL-6), and liver PGE_2 biosynthesis was measured in rats fed varying ratios of (n-6) and (n-3) PUFA. Their results conclude that the type of PUFA does not effect CLA reduction of basal levels of TNF, but does influence the effect of CLA on basal and LPS-induced IL-6 production. The authors also suggest that the type of PUFA and CLA interact to effect eicosanoid regulation of cytokine production.

Bone Metabolism

Another physiological effect of cytokines is the stimulation of bone resorption leading to weakening and wasting of bones. Since CLA modulates cytokine production, it is expected that CLA would have positive effects on bone health.

An initial study by Watkins et al. (1996) evaluated *ex vivo* PGE_2 biosynthesis, tissue IGF-I levels and bone morphometry in chicks fed diets containing either soybean oil or menhaden oil + safflower oil. Chicks fed menhaden oil + safflower oil had increased fractional labeled trabecular surface and tissue level bone formation rates compared with those fed soybean oil. *Ex vivo* PGE_2 synthesis was higher in liver homogenates and bone organ cultures from chicks fed soybean oil compared to those fed menhaden oil + safflower oil. A result of this study was that type of dietary fat modulated bone morphometry and bone PGE_2 biosynthesis. In 1997, Watkins et al. conducted a similar study as above but used different diets. Chicks were fed diets containing one of four lipid sources: soybean oil (SBO), butter + corn oil (BC), margarine + corn oil (MAC), or menhaden oil + corn oil (MEC). The results demonstrated that changes were observed in the concentration of PG precursors 20:4(n-6) and 20:5(n-3) and the ratio of SAT/PUFA. The increase in cortical bone formation in chicks fed butter suggests that the concentration of 20:4(n-6), PGE_2 precursor, is important for proper bone modeling in the young because a moderate level of PGE_2 stimulated bone formation, but a higher production of PGE_2 was associated with reduced bone formation rate.

Since these early studies suggested that fats of animal origins positively affected bone formation in chicks an additional study was undertaken to determine the effects of CLA on tissue fatty acid composition and *ex vivo* PGE_2 production in rats (Li and Watkins, 1998).

Experimental diets contained soybean oil, soybean oil + CLA, menhaden oil + safflower oil, or menhaden oil + CLA. CLA was added at 10g / kg of the diet. Dietary CLA decreased the concentrations of 16:1n-7, 18:1, total monounsaturates and n-6 fatty acids, but increased the concentrations of n-3 fatty acids (22:5n-3 and 22:6n-3), and saturates in the tissues analyzed. *Ex vivo* PGE_2 production in bone organ culture was decreased by n-3 fatty acids and CLA. The authors conclude that since CLA lowered *ex vivo* PGE_2 production in bone organ cultures, then CLA has the potential to influence bone formation and resorption.

Human Studies

The concentration of CLA in human blood and milk has been investigated to determine if dietary intake contributes to final concentration. In 1994, Yi-Chia Huang et al. fed cheddar cheese (112g of CLA/day) to nine healthy men for four weeks. Plasma CLA was significantly higher following cheddar cheese consumption than observed initially and following intervention. In an attempt to determine if linoleic acid consumption would affect plasma concentrations of CLA, Herbel et al. (1998) added safflower oil (16g LA/day) to the diets of six men and six women. Although linoleic acid intake increased significantly during the dietary intervention, plasma CLA concentrations were not affected. Also in 1998, Salminen et al., studied the dietary and serum CLA levels by comparing similar amounts of *trans* monoenoic fatty acids and stearic acid against a dairy fat-based background diet. In this study, the subjects that consumed the *trans* fatty acid diet had increased serum lipid CLA compared to the dairy fat diet. The authors hypothesize that desaturation of *trans*-veccenic acid by Δ9-desaturase remains a possible explanation for the increase of CLA in serum lipids due to a *trans* fatty acid diet.

A comparison of CLA concentrations in human milk and infant formula was conducted by McGuire et al. (1997). Conjugated linoleic acid was detectable in only 31% of formula samples but was detectable in all human milk samples. The biologically active isomer (c9, t11) in human milk ranged from 2.23 – 5.43 mg / g fat, in formula from undetectable to 2.04 mg / g fat (vegetable sources). Human milk contained significantly more CLA than did infant formula. Jensen et al. (1998) confirmed the results of McGuire et al., verifying that CLA, specifically the c9, tll isomer is present in human milk. Jensen et al. (1998) also determined that the concentration of CLA in human milk was not changed by dietary supplementation of fish oil.

Antioxidant Potential

There is growing interest in finding natural antioxidants that can prevent or treat disease. Therefore, interest in CLA is growing because it has shown potential as a protective agent against the formation of free radicals both *in vivo* and *in vitro* in cancer. Ha et al. (1990), Pariza et al. (1991), and Ip et al. (1991) have suggested antioxidant activity to explain CLA's anticarcinogenic effects. Ha et al. showed that CLA was more potent than α - tocopherol and as effective as butylated hydroxytoluene (BHT) in inhibiting iron-thiocyanate-induced peroxide formation. The *in vivo* rat study by Ip et al. (1991) demonstrated that CLA was as effective as vitamin E and BHT in inhibiting the formation of TBARS (thiobarbituric reactive substances). TBARS are often used to assess oxidation in biological systems. These results by Ip directly link dietary CLA, oxidation, and mammary carcinogenesis. Lee et al. (1994) also suggested that antioxidant properties of CLA may be involved in reduced atherosclerosis of rabbits.

van den Berg et al. (1995) regarded these early studies as ambiguous. Their study tested whether CLA (control, .75 μM, 25 μM and 50 μM) could protect membranes composed of 1-palmitoyl-2-linoleoyl phosphatidylcholine (PLPC) from oxidative modification under conditions of metal ion-dependent or independent oxidative stress. van den Berg et al. (1995) concluded that CLA, under these conditions, did not act as a free radical scavenger. Therefore, they concluded that CLA as an antioxidant does not seem plausible. Chen et al. (1997) investigated the antioxidant or prooxidant effects of CLA in canola oil. Their results indicate that free CLA and CLA methyl ester accelerated lipid oxidation in canola oil. This prooxidant activity was dose-dependent at concentrations ranging from 0.1 to 1.0% canola oil. CLA-containing triacylglycerol, however, was neither an antioxidant nor a prooxidant. These authors concluded that CLA was not an antioxidant in fats and oils. Banni et al. (1998) evaluated the oxidative potential of CLA using fatty acid analysis of liver homogenate, oxidized *in vitro* either with Fe-ADP or t-butyl hydroperoxide (t-ButylHP) of lambs and rats. Results showed that CLA and its metabolites steadily decreased during oxidative stress and were more prone to oxidation than their methylene-interrupted fatty acids. Banni concluded that CLA effects are not related to interference in the lipoperoxidative processes.

Chemistry and Analytical Methods

Early methods used to quantitate conjugated acids were based on ultraviolet spectroscopy. CLA absorbs between 230 and 235 nm (Reil, 1963, and Dormandy and Wickens, 1987). Complex mixtures of fatty acids which contain other conjugated fatty acids

can interfere with the absorbance of CLA resulting in inaccurate results (Shantha et al., 1993).

GC methods have been used to quantitate CLA isomers. Typically this analysis has used analysis of fatty acid methyl esters (FAME). Several different methods have been used to prepare FAME including, acid-catalyzed methylation (Shantha et al., 1992), base-catalyzed methylation (Aneja and Murthi, 1991), and AOCS (1) official method which involves both basic and acid reagents (Ha et al., 1989, Ha et al., 1987, Ha et al., 1990, and Ip et al., 1991). The official AOCS (1) method for methylation clearly states that this method is not applicable for conjugated polyunsaturated fatty acids because the reagents (alcoholic sodium hydroxide and boron triflouride in methonal) cause the double bonds to become unstable upon fragmentation and to migrate along the carbon chain (Dobson and Christie 1996). The AOCS (2) method often used to quantitate CLA isomers does not have the same limitations (Ha et al., 1989, Ha et al., 1987, Ha et al., 1990, and Ip et al., 1991).

In an attempt to obtain higher yields of FAME, Shantha and Decker (1993), used boron triflouride-methanolic sodium hydroxide and Aneja and Murthi (1991) used sodium methoxide in methanol as methylating reagents. Shantha concluded that BF_3-MEOH was not suitable for use in CLA quantitation because *cis*, *trans*-conjugated fatty acids were lost in artifact form. These authors also concluded that direct tranesterification with methanol-benzene-acetyl chloride (DAC) was not suitable for CLA quantitation because of a drastic increase in the *trans*, *trans* CLA isomer and the formation of an artifact. After comparing methods mentioned above Shantha and Decker (1993) agreed with Aneja and Murthi (1991) that mild basic methylating reagents such as NaOMe-MeOH are acceptable for analysis of CLA in food samples with low concentrations of or no free fatty acids.

Yurawecz et al. (1994) studied a mixture of allylic hydroxy oleates (AHOs) that were tested by methylation procedures with several catalysts: BF_3, HCl, NaOMe, and tetratmethylguanidine. AHOs are secondary products of lipid autoxidation and their ability to be converted to CLA during certain methylation procedures could produce unrealistically high CLA concentrations. In this study, Yurawecz found that both acid-catalyzed methylation procedures, BF_3 and HCl converted AHOs to CLA. However, both base-catalyzed methylation procedures, NaOMe and tetramethylguandidine did not convert AHOs to CLA. The authors suggest that care should be taken when reviewing early CLA results if acid-catalyzed methylation was used to determine CLA concentrations.

Banni et al. (1994), used a combined HPLC / second-derivative ultraviolet analysis with on-line atmospheric-pressure ionization mass spectrometry (MS) to determine whether fatty acids with conjugated dienes present in partially hydrogenated vegetable oils were CLA. These authors concluded that indeed partially hydrogenated vegetable oil fatty acids with conjugated dienes are isomers of linoleic acid. They suggest that partially hydrogenated fats such as hydrogenated soybean oil and margarine should be added to the list of foods known to contain CLA.

In 1998, Sehat et al. reported that silver-ion high-performance liquid chromatography (Ag+-HPLC) clearly separated four isomers, 11c, 13t-, 8t, 10c-, 9c. 11t-, and 10t, 12c-18:2. These four isomers were found in a commercial CLA preparation. In a related study, Kramer et al. (1998) identified another problem with early CLA quantitation, which was a lack of chromatographic methods to separate all the individual CLA isomers. These authors used both GC and Ag+-HPLC to separate CLA as fatty acid methyl esters (FAME) from pig tissues. Major CLA isomer identity was confirmed by GC-direct deposition-Fourier

transform infrared spectroscopy and GC-electron ionization mass spectrometry. Kramer suggested that optimal conditions and a long polar GC column (100m SP-Sil 88) were able to separate 10 CLA peaks. Even under these specific conditions some isomers coeluted and could not be identified without the Ag+-HPLC method.

Lavillonniere et al. (1998), confirmed that GC analysis alone did not individually resolve all CLA isomers. They also suggest that the use of methyl ester derivatives of CLA are inappropriate for determination of double-bond position as did Dobson and Christie (1996). In this study they combined GC-Fourier transform infrared spectroscopy and GC-MS of dimethyloxazoline derivatives (DMOX) to determine both geometry and double-bond location of CLA in French cheeses. Using these methods they determined that 14 isomers were present in cheeses and that the 18:2Δ9c,11t was the main CLA isomer in these cheeses.

In summary, the method described by Kramer et al. (1998) and Sehat et al. (1998) appears to be the most complete method available to identify and quantify CLA isomers.

Conjugated Linoleic Acid in Animal Products

Milk and Milk Products

Milk. Dietary sources of CLA include milk fat, meat products, and vegetable oils. Animal sources contain more CLA than vegetable sources, and foods of ruminant origin contain more CLA than do foods of nonruminant origins. Milk and dairy products are therefore one of the major dietary sources of CLA. In 1955, Shorland et al. demonstrated that CLA is produced from polyunsaturated fat by the rumen microorganism. Kepler and Tove (1967) showed that the first intermediate in the biohydrogenation of linoleic acid by rumen bacteria, *Butyrivibrio fibrisolvens*, is c-9, t-11-18:2.

Initial study of conjugated octadecadienoic acid began after Booth et al. (1935) noted that in milk fat fatty acids with conjugated unsaturation in cows turned out to pasture greatly increased the absorption in the ultra-violet at 230 nm. Mattsson (1949) using a spectrophotometric method quantitatively measured this change. A review by Riel (1963) summarized CLA concentrations evaluated by spectrophotometric methods indicating a range of 2.4 to 21.8 mg/g fat. Each of these studies noted that diet and individual cow variation greatly effected concentration variation.

The effect of lactation number (age) on PUFA content and oxidative stability of buffalo and cow milk was investigated by Lal and Narayanan (1984). The average content of dienoic acids of buffulo and cow milk fats decreased slightly from 1st lactation group (1-3 lactations) to 2nd lactation group (4-6 lactations) and then increased in the 3rd lactation group (7 or more lactations). They also noted that the same pattern existed in peroxide deveopment. Overall, they concluded that lactation number has a slight effect on PUFA and oxidative stability of milk.

Lin et al. (1995) surveyed the conjugated linoleic acid contents of numerous dairy products. Fifteen cheeses, three fermented dairy products, and four fluid milk products were tested. The CLA content of cheeses ranged from 3.59 – 7.96 mg / g of lipid. Significantly higher CLA content was noted in Blue, Brie, Edam, and Swiss than other cheeses. The authors suggest that this increase was due to elevated levels of incorporated oxygen which enhanced free radical oxidation of linoleic acid to CLA. The CLA content of other fermented dairy products ranged from 3.82 – 4.66 mg / g of lipid, with cultured buttermilk having the highest content. The CLA content of fluid milk ranged from 3.38 – 6.39 mg / g of lipid.

These results along with those of Ha et al. (1987) verify that dairy products are the major source of CLA in the human diet.

Several recent studies have attempted to define the effects of different feed types and dietary regimens as methods to enhance CLA concentrations in milk. Jiang et al. (1996) divided cows into three dietary groups. The control group was fed a 50:50 forage to concentrate ratio. The two treatment groups were fed a trial diet with either restricted or unrestricted amounts. Their results demonstrated that there was a large variation in the c9, t11 isomer in raw milk and this variation can be influenced by diet. Stanton et al. (1997) compared the effects of grass dry matter allowance and full fat rapeseed supplementation on levels of c9, t11 CLA isomers. Full fat rapeseed supplementation resulted in increases in milk CLA, however reduced levels of grass intake caused decreases in milk CLA. The effects of buffers (1.1% $NaHCO_3$ + 1.1% $KHCO_3$, 1.9% $NaHCO_3$, 0.5% MgO, and 2.0% Na sesquicarbonate) on milk fatty acids and mammary arteriovenous differences in dairy cows fed calcium salts of fatty acids was studied by Thivierge et al. (1998). Their results indicate that buffers increased $C_{18:2}$ in milk fat, but MgO decreased $C_{18:2}$ and $C_{18:3}$ and increased *cis*-$C_{18:1}$.

Kelly et al. (1998) studied the effects of fresh pasture on concentration of CLA in milk fat. When cows were consuming a diet consisting of pasture only, CLA concentrations in milk fat doubled (10.9 vs 4.6 mg / g of milk fat). Individual consistency of CLA content of individual cows was demonstrated although between cow variation was large. In another study by Kelly et al. (1998) forage was combined with peanut oil, sunflower oil, and linseed oil. Conjugated linoleic acid concentrations (mg / g of milk fat) were 13.3, 24.4, and 16.7 during peanut oil, sunflower oil, and linseed oil treatment, respectively. This study

concluded that CLA concentration in milk fat can be enhanced by the addition of oils high in linoleic acid. These studies taken together suggest that dietary manipulation can increase CLA concentrations in milk fat.

Milk Products. Historically, fluid milk has been identified as a food rich in CLA. Dairy products are good sources of CLA, and a considerable amount of research has been done on the CLA content and isomer distribution in milk products. Ha et al. (1989) determined that fat in cheese normally had greater concentrations of CLA than did milk. The increased concentrations in cheeses is most likely due to the removal of water during processing, thus increasing the lipid percentage. Ha associated these differences to two parameters: 1) isomerization of linoleic and linolenic acids in the rumen and 2) free-radical oxidation of linoleic acid caused by aging, heat treatment, and protein quality. This study measured CLA content in existing retail products and did not control processing practices. The studies that followed identified how cultures, processing, aging, storage and various antioxidants effected the CLA content of various cheese or processed cheese products.

To evaluate the concentrations of CLA in various cheese products Shantha et al. (1992) bought cheddar cheese and different varieties of processed cheese from a local grocery store. They also studied the effect of whey protein concentrate (1.5, 3.0, 4.5, or 6.0%) on CLA formation in processed cheese. The results of this study indicate that both processing conditions and whey protein concentrate affect CLA concentration in processed cheese. Also in 1992, Werner et al. evaluated the effects of different cultures, processing conditions, and aging periods on three 13-month-aged and one unaged cheddar type cheeses. The CLA concentration in all types of cheese ranged from 5.05 to 5.39 mg CLA / g of fat. There were four isomers detected (c9, t11-, t9, c11-, t9, t11-, and t10, t12-). The c9, t11- and

t9, c11- isomers accounted for 82 - 88% of the total CLA and the t9, t11- and t10, t12- isomers accounted for 8 - 11% of the total CLA. The authors concluded that different starter cultures, processing conditions, and aging periods had little effect on total CLA concentration but did influence CLA isomer distribution in the cheeses studied.

Several hydrogen donors (butylated hydroxytoluene, propyl gallate, cysteine, ascorbic acid) and dairy additives (sodium caseinate, sweet whey powder and nonfat dry milk) were added to processed cheese by Shantha and Decker (1993) to determine their effects on CLA concentration. The most effective hydrogen donor tested was propyl gallate which increased the total and c9, t11- CLA content by 1:59 and 1:42-fold. Sodium caseinate was the most effective dairy derived additive, increasing CLA concentration 1:65-fold compared to processed cheese with no additive. Also, addition of ferrous ions did increase CLA concentrations, suggesting that iron-catalyzed free radical initiation does increase CLA concentrations. Although CLA concentration was increased by each category studied, a large amount of the original linoleic acid was not converted to CLA during processing. Therefore, the authors suggest that other limiting factors are involved in CLA formation perhaps related to the cheese-making process and / or isomerization of CLA.

In 1995, Shantha et al. evaluated the effects of production practices in manufacture of yogurt, sour cream, ice cream, butter, and cheeses on CLA concentrations of finished and stored products. Dairy product such as ice cream, sour cream and cheeses (Mozzarella, Gouda and Cheddar) showed no increase in CLA due to processing. The total CLA content of both salted and unsalted butter increased, as did the CLA content of nonfat yogurt. Although CLA concentrations did not increase during processing of most dairy products it did not decrease, suggesting that CLA is a stable component.

Factors such as packaging type (cans and nylon vacuum pouches), milling pH (5.5, 5.7, and 5.9), additives [butylated hydroxyanisole (BHA), tyrosine, and lysine], and aging (0, 1, 3, and 6 months) were used to determine conjugated linoleic acid concentrations in cheddar cheese by Lin et al. (1998). Their results indicate that the CLA content in canned cheese (3.03 mg / g lipid) was significantly higher than in a vacuum pouch packed cheese (2.70 mg / g lipid) after 6 months of aging. The authors suggest that lipid oxidation enhanced radical formation in canned cheese, which could then be converted into CLA through protonation of the radicals. Significantly lower CLA contents were found in cheeses aged 6 months that had milling pHs of 5.5 and 5.9, and had BHA, tyrosine, and lysine added. A multiple linear regression showed that both protein and lipid content contributed positively to CLA content.

Conjugated linoleic acid in meat

Chin et al. (1992) conducted a comprehensive study of the CLA concentration of various foodstuffs. They found that the principal dietary sources of CLA were animal products. Overall, meats from ruminants contained more CLA than did meat from nonruminants. Lamb contained the greatest amount of CLA (5.6 mg / g fat) in ruminants while veal had the least amount (2.7 mg / g fat). Beef products ranged from 2.9 mg / g fat in round to 4.3 mg / g fat in fresh ground beef. Nonruminants had less CLA than did ruminants. Pork (0.6 mg / g fat) and chicken (0.9 mg / g fat) were quite low, yet turkey had a much higher concentration (2.5 mg / g fat). CLA content of seafood ranged from 0.3 to 0.6 mg / g fat. The c9, t11 CLA isomer accounted for 76% of total CLA in ruminants. Dairy products were generally high in CLA content. Cheese ranged from 2.9 to 7.1 mg/g fat of CLA. The c9, t11 CLA isomer accounted for 83% of the total isomers in natural cheeses. Plant oils also

contained less CLA (0.1 to 0.7 mg / g fat) than did animal fats. Also the distribution of isomers in plant oils was different than in animal fats. The c9, t11 and t10, c12 isomers accounted for 43 and 40% of the total CLA, respectively. This study concluded that since CLA is found mainly in ruminants it would be of interest to determine if dietary intake would influence CLA concentrations in the tissues of nonruminants.

The effects of cooking temperatures, cooking methods, and refrigerated storage on beef steaks (ribeye, round, T-bone, and sirloin) and ground beef to determine CLA concentrations was studied by Shantha et al. (1994). This study reported that there was a wide variation in CLA content (3.1 to 8.5 mg CLA / g fat) of raw steak on an animal to animal basis which therefore produced no significant differences in CLA between cooked steaks. No differences were found for either cooking methods or degrees of doneness when results were compared on a milligram of CLA per gram of fat basis. When patties were compared on a milligrams per 100 grams of cooked meat basis, the 80 °C baked patties had the highest CLA concentration (152 mg/100 g meat), while baked patties cooked at 60 °C contained the greatest concentration of CLA on a per patty basis (113 mg of CLA/ patty). The authors noted that all cooked beef oxidized rapidly during storage; however, oxidation did not effect CLA concentration.

Animal Performance and Body Composition

Chin et al. (1994) conducted the first report that CLA may have positive effects on performance and feed conversion. Conjugated linoleic acid (0.25% or 0.5%) was fed to female Fischer rats during gestation and/or lactation. CLA was incorporated into both milk fat and tissue lipids in amounts proportional to those fed. Conjugated linoleic acid was also incorporated into fetal and neonatal tissue. Feeding CLA to the dams significantly increased

the postnatal body weight gain of pups. Pups given CLA post weaning also had significantly greater body weight gain and improved feed efficiency compared to the controls.

In 1996, Banni et al. evaluated the CLA concentrations in milk, dairy products, liver and adipose tissue of lambs. Liver fatty acid, phospholipid and neutral lipid analysis indicated three CLA isomers were present. They found that CLA was converted into conjugated diene arachidonic acid, and that the conjugated diene arachidonic acid was incorporated into the tissue phospholipids. This suggests that conjugated arachidonic acid may compete with arachindonic acid in the biosynthesis of eicosanoids. These authors concluded that conjugated linoleic, eicosatrienoic, and arachidonic acids were detected in lamb phospholipids demonstrating that elongation and desaturation does occur in lamb tissues.

Although the initial study involving performance and feed conversion was in rats the next such study was in pigs. The study by Dugan et al. (1997) fed 108 pigs (54 barrows and 54 gilts) either 2% CLA or 2% sunflower oil. The results suggested that CLA fed pigs tended to have reduced feed intakes and improved feed conversion. Also, pigs fed CLA had less subcutaneous fat and more lean than pigs fed sunflower oil. The authors conclude that feeding CLA to pigs repartitions nutrients from fat toward lean deposition. In a similar study, Dunshea et al. (1998) fed CLA (0, 1.25, 2.5, 5.0, 7.5, and 10.0 g / kg) to 30 gilts. CLA was substituted for soy oil in the diet. Dunshea reported that CLA had no effect on feed intake, however average daily gain and feed conversion efficiency tended to be increased by CLA supplementation. Final pig weight also tended to be higher from CLA fed pigs. Dietary CLA reduced P_2 backfat at all levels of supplementation (21.0, 17.1, 16.1, 16.9, 15.4 and 14.6 mm, respectively).

An earlier discussion of dietary manipulation to increase CLA in milk fat has implications for increasing CLA concentrations in beef muscle. To determine if the c9, t11 isomer of CLA could be found in semimembranosus muscle of cattle, Shantha et al. (1997) fed cattle on pasture (without grain added), grain on grass, and implanted animals. The c9,t11 isomer of CLA was higher in pasture fed animals (7.4 mg / g fat) than in grain and grass fed (5.1 mg / g fat) or implanted (5.2 mg / g fat) cattle. The authors noted that linoleic acid was found in lower concentrations in grass-fed animals (23.2 mg / g fat) than in grain on grass fed (26.6 mg / g fat) and implanted (27.5 mg / g fat) animals. They suggest this decrease in linoleic acid may be due to conversion to CLA.

Park et al. (1997) studied the effect of CLA on body composition in mice. ICR mice were fed diets containing corn oil (5.5%) or a CLA-supplemented (5.0% corn oil + 0.5% CLA). The CLA fed mice exhibited lower body fat (57% in males, 60% in females) and increased lean body mass (5% in males, 14% in females) compared to controls. Included in this study was an analysis of cultured 3T3-L1 adipocytes. In this culture CLA significantly decreased heparin-releasable lipoprotein lipase activity (-66%), intracellular triacylglyceride concentrations (-8%) and glycerol (-15%), but increased free glycerol (+22%) compared to controls. These results taken as a whole suggest that the effects of CLA on body composition is due in part to decreased fat deposition and increased lypolysis.

The most recent study by Cook et al. (1999) investigated fat reduction in pigs. Twenty-four pigs were fed diets containing 0. 4.8, or 9.5 g CLA-60 / kg diet. Feed intake was decreased over the first 49 days of CLA supplementation however, feed intake recovered for the remainder of the trial. Tenth rib backfat thickness was reduced 24% for CLA supplemented pigs over controls. No differences were reported for final carcass weight or

loin muscle area. An increase in Minolta L* value indicated that lean color was darker for CLA fed pigs. Loin dissection data tended to show less fat (intermuscular and subcutaneous) and showed a significant increase in lean for CLA fed pigs compared to controls.

Sensory Evaluation

According to Reume (1975), "sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing. " The sensory characteristics of foods are a combination of appearance attributes, odor or aroma, textural attributes, and flavor. Appearance is the color, size and shape of the sample (Meilgaard et al., 1991). Textural attributes (kinesthetics) of a sample include texture, viscosity and mouth feel (Meilgaard et al., 1991). Flavor is a combination of the tastes, aromatics and chemical feeling factors related to the four basic tastes; sweet, sour, salty and bitter (Meilgaard et al., 1991).

The concept of a sensory panel is to use the panel as a measuring device, which is analogous to the use of any scientific instrument. For this reason, panelists should be trained and actively involved in the development of scales used to measure panelist response (Meilgaard et al., 1991). There are four basic types of tests designed to use with trained panelists, they are difference tests, ranking, scaling, and descriptive analysis. Difference tests are used to determine whether two foods can be distinguished from each other based on their sensory characteristics. In ranking tests, three or more samples are presented at the same time and arranged in order of intensity of a specified attribute. Scaling methods use numbers or words to express the intensity of a specified attribute. Descriptive analysis is

used to characterize the component sensory attributes in complex stimulii. The results of most sensory panels reported in the literature is intended to be of a descriptive nature because the researcher is interested in the effects of the studied variables on the food (Meilgaard et al., 1991).

Sensory evaluation is an important measurement in meat research. The majority of sensory panel tests reported in meat science research include hedonic rating, scoring and preference/acceptance tests. Hedonic rating measures the level of like or dislike that a panelist has for a specific attribute or the product in general (Meilgaard et al., 1991). In scaling the intensity of a specific attribute is indicated by a mark on a rating scale or by a numeric score (Abbott, 1973, and Meilgaard et al., 1991). Many researchers prefer scales without numbers because they feel that it creates the impression of a continuum and gives panelists a means of locating a sample on the continuum without bias (Stone et al., 1974).

There are many psychological /environmental factors, which can effect sensory panel results in food. The room should be quiet, free from interruptions and distractions (Meilgaard et al., 1991). Panelists will always find what they expect to find and will use all available information (relevant and irrelevant) to make a decision. Panelists will also be influenced by each other and will respond to their total surroundings. In addition, if several characteristics are to be evaluated at one time the Halo effect may occur, in which one characteristic influences the others (Meilgaard et al., 1991). In order to eliminate many of these problems certain criteria for testing environment and panelists must be used. The sensory panel facilities should be accessible, free from distractions, have good lighting and in general be comfortable. Meilgaard et al. (1991), suggests that panelists do not eat one hour prior to panel, not smoke one hour prior to panel, avoid the use of perfume, and not

participate in panels when ill. Factors related to sample preparation and service should also be controlled. The uniformity of samples presented to panelists should include appearance, sample size, temperature, coding, order, and palate rinsing. Food should be tested under conditions approximately the same as normal consumption (Meilgaard et al., 1991).

Dietary Lipids on Pork Sensory Characteristics

Due to the amount of unsaturated fatty acids in pork adipose tissues and the ability of those adipose tissues to be modified by dietary lipid supplementation an important issue is the effect of dietary lipids on sensory and / or quality characteristics of pork. In 1926, Ellis and Isbell showed that linoleic acid was readily incorporated into fat tissues but at high levels caused processing problems due to "soft fat". Several studies reviewed by Wood (1984) from the United Kingdom found that the causes of soft fat were related to a critical reduction in lipid melting point. A melting point above 15g linoleic acid / 100g total fatty acids of backfat indicated sufficient triacylglycerols contained both linoleic and oleic acid. To obtain acceptable melting points the diets required 16g linoleic acid / kg.

The evidence that monounsaturated fatty acids do not raise blood cholesterol levels has led some researchers to explore the feeding of diets rich in oleic acid to pigs (Wood and Enser, 1997). Rhee et al. (1990) fed 12% high-oleic sunflower oil, which increased the oleic acid concentration in muscle tissue from 42-53g / 100g. The high-oleic sunflower oil significantly increased juiciness, muscle fiber tenderness and overall tenderness scores than those from controls. In addition, Rhee et al. (1990) reported significantly lower shear values and no differences in off-flavor intensity, overall flavor, and oiliness scores between sunflower oil treatments and controls.

Another study using high levels of dietary oleic acid from sunflower seed oil by Shackelford et al. (1990) evaluated sensory characteristics of ham. Even though the concentration of oleic acid increased to 52g / 100g, sensory characteristics were not improved. Shackelford et al. (1990) also fed low euric acid rapeseed oil, which increased the concentration of α - linolenic acid to 3g / 100g in muscle lipid. This increase was related to the ability of panelists to detect off flavors in cured ham served cold. High proportions of panelists (65%) were able to detect off-flavors in bacon. Shackelford et al. (1990) suggested that this was directly due to the increased concentrations of linoleic acid derivatives (2-pentenol and 2,4-heptadienal) formed during processing.

Pigs have also been fed peanuts which increased the ratio of unsaturated to saturated fatty acids in backfat. Ellis (1933) and Alsmeyer et al. (1955) determined that pigs fed peanuts for extended periods of time developed carcasses with soft, oily fat. These detrimental effects were verified by Myer et al. (1985) who found these effects proportional to the amount of weight gained while pigs gleaned peanuts. West and Myer (1987) determined that feeding peanuts for part or all of the pigs weight gain did not influence carcass characteristics or meat quality characteristics, with the exception of a decrease in subjective marbling. Palatability of broiled loin chops and frozen stability of loin sections from these carcasses were also not affected.

A potential benefit of feeding diets rich in α - linolenic acid is that increased deposition could lead to increased synthesis of the longer-chain polyunsaturated fatty acids, 20:5 (eicosapentaenoic acid) and 22:6 (docosahexanoic acid) which are n-3 fatty acids (Wood and Enser, 1997). An adequate intake of both 20:5 and 22:6 may prevent adult diseases such as atherosclerosis and heart disease (Lands, 1986). The n-3 polyunsaturated

fatty acids (PUFA) such as 20:5 and 22:6 are found in abundance in fish oil but are scarce in animal fat (Irie and Sakimoto, 1992). Banks and Hilditch (1932) and Garton et al. (1952) observed an increase in the content of C20 and C22 PUFA in pigs fed fats of marine origin. Irie and Sakimoto (1992) observed that when fish oil was supplemented at 2, 4, or 6% of the swine diet, increased n-3 PUFA was found in all carcass fats measured. Irie and Sakimoto (1992) used Hunter L, a, and b values to determine that fat color showed no evidence of yellow fat. Iodine number and refractive index increased linearly with supplementation of dietary fish oil demonstrating both softer fat and more potential for rancidity.

Measurements of Pork Carcass Composition

Variation in composition of pork carcasses is affected by numerous factors including breeding, diet and marketing strategies. For approximately 75 years, investigators have wanted to determine differences in carcass characteristics without sacrificing the carcasses. The desire to find simple, nondestructive indicators of composition has been dealt with extensively in the literature.

One of the most direct classical methods of determining the whole body composition of pigs is complete physical separation. The first observations recorded in which each organ and tissue were individually analyzed was by Laws and Gilbert (1859). They physically dissected and performed chemical analysis on three beef carcasses, five lamb carcasses and two pork carcasses. Many studies have been undertaken in which complete dissection of carcasses has been done. Complete carcass dissection is typically done when detailed information about various tissue is required (McMeekan, 1941, and Callow, 1947). Callow (1962), indicated that there are no adequate shortcuts to whole side or whole carcass

dissection. When meticulously carried out the separation technique can unquestionably yield valuable information in terms of the ratio or increase of the separated entities (Zobrisky, 1963). Total carcass or side dissection is time consuming, labor intensive and expensive therefore, a great deal of effort has been directed toward the selection of portions of the carcass which are representative of the whole. The separable components of both the ham and loin (Hankins and Ellis, 1934, McMeekan, 1941, Aunan and Winters, 1949, Zobrisky 1963, and Adam and Smith, 1966) have been reported to be the most reliable indicator of pork carcass composition.

Many different measurements on exposed cut surfaces have been investigated in an attempt to determine which measurements would most reliably indicate composition. Hammond (1932) proposed a growth gradient theory in lamb, in this theory the loin is the latest maturing cut. Subsequent studies by his lab demonstrated that overall muscle development could be estimated most effectively by measuring the cross-sectional area of the longissimus muscle at the last rib. From that point on, area of the longissimus muscle has been used extensively as an index of muscling (Hedrick, 1983).

A series of methods have been employed to determine the most accurate and rapid measurement of the longissimus area. The first attempt was made by Mackintosh (1937) when he placed a piece of parchment paper on the exposed longissimus muscle and traced the muscle outline on the paper. These outlines were then measured with a planimeter. In 1953, Stull used a camera to photograph the longissimus muscle of beef at the 12^{th} rib. The photographic technique of Stull was modified by Schoonover and Stratton (1957) by placing a wire grid over the cut muscle surface and then taking the photograph. Shrewsbury and Wideman (1961) photographed both the muscle and adjacent fat area and measured both

areas with a planimeter. Four different methods of measuring longissimus area were investigated by Henderson et al. (1966). The four methods tested were counting grids .645 cm^2, counting dots in the center of a .645 cm^2 area, measuring a tracing of the muscle and measuring the projection of a 35-mm transparency. Of these four methods Henderson determined that tracing the cross-sectional area with a planimeter was the most precise method. Henderson (1966) also concluded that although differences were observed between the four methods, these differences were small compared to the differences in longissimus muscle area between right and left sides. Because these differences between methods were small, the relative speed and ease of using the dot and grid method has made them the most popular method for measuring carcasses.

Over the years, various locations along the longissimus muscle have been measured and then compared to carcass composition. In the United States, longissimus area is currently measured between the 12^{th} and 13^{th} ribs for beef and lamb and between the 10^{th} and 11^{th} ribs for pork. Aunan and Winters (1952) determined that the ratio of fat to lean tissue in 5-6 rib samples was highly associated with the ratio of fat to lean tissue of the carcass. Murphy et al. (1960) reported that prediction equations including longissimus muscle area were excellent indexes of muscling. Bowman et al. (1962) reported that measurements of fat and lean at various cross sections, particularly at the third lumbar, were appreciably better than routine carcass measurements. Although the third lumbar was the best predictor of carcass fat and lean the tenth thoracic was a very close second. In a companion study by Bowman (1962), cross sectional tracings were taken at eight points. Their results indicated that area measurements of fat and lean were most accurately obtained in the mid region (loin) of the carcass compared to either extremity (shoulder, ham). By 1976 the NPPC procedures

to evaluate market hogs used 10^{th}-11^{th} rib loin eye area as one of three factors to be used as an accurate and practical standard to objectively measure pork carcass composition (Fahey et al. 1977). NPPC (1991) continues to recommend the use of loin muscle area between the 10^{th} and 11^{th} rib as a practical measurement of carcass composition.

Numerous reports have consistently demonstrated that fat is the most variable component of the carcass. When the percentage of fat in a carcass increases, there is an almost equal decrease in lean. In an attempt to find which measurements were most highly associated with carcass composition both subcutaneous and intermuscular fat measurements at almost every possible location have been taken.

Scott (1927) demonstrated that variations found in the percentage of ham, loin and other pork cuts could be directly related to the fatness of the pork carcass. These results were confirmed by McMeekan (1941) using physical separation of the carcass into lean and fat. The importance of backfat thickness in pork carcass evaluation has been investigated by many researchers (Hankins and Ellis, 1934; Aunan and Winters, 1949; Zobrisky et al. 1954). These studies demonstrated that backfat thickness is one of the most practical measurements obtainable for estimating the amount of fat. The common slaughter practice to split the carcass in half along the midline allows several measurements to be made either individually or in combination along the exposed surface. Caution must be used when using these measurements alone to indicate amount of fat due to the potential for uneven splits. Two groups of researchers (Evans and Kempster, 1979; and Edwards et al. (1981) have suggested that the fat measurements taken over the longissimus muscle at approximately three-quarters of the distance from the medial side of the muscle are more accurate measurements than those taken from the split surface. The NPPC procedures to evaluate market hogs (1991)

includes fat depth (including skin) over the loin at the 10^{th} rib and last rib fat thickness as simple fat measurements used to estimate carcass composition.

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CONJUGATED LINOLEIC ACID EFFECTS ON SWINE PERFORMANCE AND CARCASS COMPOSITION

A paper to be submitted to the Journal of Animal Science

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ABSTRACT

Conjugated linoleic acid (CLA) is a collective term for positional and geometric isomers of linoleic acid. The effect of dietary CLA has been shown to improve feed efficiency, decrease body fat and increase lean composition. We hypothesized that CLA would improve performance and carcass composition, and would be deposited in pork tissues. Diets of 40 crossbred pigs were supplemented with conjugated linoleic acid to determine its effects on performance and carcass composition. Eight replications of five littermate barrows with an initial average weight of 26.3 kg were allotted at random to individual pens. Within replication dietary treatments containing 0, .12, .25, .5 or 1.0 % CLA were assigned at random. Pigs were weighed and feed disappearance was determined at 14-day intervals. Average daily gain increased linearly as the level of CLA increased in the diet ($P < .05$). Average daily feed intake was not affected by the concentration in the diet, therefore, there was a linear improvement in gain to feed ratio ($P < .05$). Carcasses from animals fed control diets had more tenth rib backfat than carcasses from animals fed CLA ($P < .05$). Ultrasound measurement and carcass measurements showed a decrease in fat depth over the loin eye at the tenth rib of CLA treated pigs ($P < .05$) than observed for control pigs. Belly hardness (firmness) increased linearly as the concentration of CLA in the diet increased when bellies were measured for firmness either lean side up ($P < .001$) or lean side down

($P < .05$). Loin dissection data demonstrated that CLA produced a quadratic treatment effect for both less intermuscular fat ($P < .001$) and subcutaneous fat ($P < .05$) and a linear increase for bone ($P < .05$), although finished loin weight only tended to increase ($P = .08$). CLA concentration increased in a linear relationship in both subcutaneous fat ($P < .001$) and lean tissue ($P < .001$) as measured by gas chromatography of the CLA. Dietary CLA was incorporated into pig tissues and had positive effects on performance and body composition.
Key Words: Conjugated Linoleic Acid, Pork, Body Composition

Introduction

Conjugated linoleic acid (CLA) consits of a group of positional (double bonds in positions 9 and 11 or 10 and 12 on the carbon chain) and geometric (*cis* or *trans*) isomers of linoleic acid. Dietary sources of CLA include milk fat, meat products, and vegetable oils. Chin et al. (1992) found CLA in a wide variety of foodstuffs with meat and milk from ruminants having the highest content. CLA has been reported to have potent repartitioning and feed efficiency improving effects in rats (Chin et al., 1994). Mice fed CLA were found to have significant changes in body composition, including decreased fat deposition, increased whole body protein and increased carcass water (Park et al., 1997). Dugan et al. (1997) reported increased lean and decreased subcutaneous carcass fat when CLA was fed to pigs. In addition, CLA has been shown to stimulate the immune system (Cook et al., 1993) and protect against chemically induced cancers (Ip et al., 1994, Ip et al., 1997 and Belury et al., 1996) and atherosclerosis (Lee et al., 1994 and Nicolosi et al., 1997). Because of the recent availability of food grade CLA, experimentation on large animals is now possible. As a result, considerable interest for including CLA in pig feed to improve lean production

efficiency, pork quality and provide value-added healthful meat products for human consumption now exists. The objective of this study was to determine the growth performance, body composition and tissue deposition of CLA in growing-finishing pigs fed diets containing 0, .12, .25, .50 and 1.0% CLA by weight of the diet.

Materials and Methods

Animals and Diets

Eight replications of five littermate barrows (Yorkshire x Landrace x Duroc x Hampshire) with an average initial weight of 26.3 kg were allotted at random to individual pens. Within replication, treatments of diets containing 0, .12, .25, .50, or 1.0% CLA were assigned at random. The CLA source (PharmaNutrients, Oak Brook, IL) contained 60.5% CLA (Table 1) and was added to the diets at concentrations of .20, .42, .83, or 1.67% to provide the desired concentration of CLA. The conjugated linoleic acid was substituted for corn. The diets were initially formulated to contain 18.7% crude protein and 1.0% lysine (Table 2). Diets were reformulated at about 3 wk intervals (six formulations) to reduce crude protein and lysine content to 12.3% and .55%, respectively, in the final finishing stage to meet NRC requirements. Room temperature was maintained at about 18 - 21°C. Pigs were allowed ad libitum access to feed and water. Individually housed pigs were weighed and feed disappearance was determined at 14-day intervals.

Body and Carcass Composition

Ultrasound was used to determine backfat thickness and loin eye area (LEA) at approximately 52, 68, 91, and 114 kg body weight. These measurements were used to determine at what live weight control and CLA fed pigs first exhibited differences in backfat

thickness and loin eye area. Scanning was accomplished with an ALOKA 500V (Corometrics Medical Systems, Wallingford, CT) real-time ultrasonic machine fitted with a 12.5 cm long, 3.5 Mhz linear array transducer. Ultrasound measurements were taken along the dorsal midline at the tenth rib. The transducer was aligned perpendicular to the spine at the tenth rib. Digitized images were interpreted using Quality Evaluation and Prediction (Iowa State University, Ames, IA) a computer software package developed specifically to measure linear distance and area of digitized images and matriculate to a data file. Tenth rib backfat was measured as the distance from the outer edge of the skin to the start of the fascia layer in the center of the longissimus muscle at a point approximately 6.35 cm lateral to the spine.

Pigs were slaughtered when the average weight of littermates was 116 kg according to humane slaughter requirements at the Iowa State University Meat Laboratory. Post slaughter, the carcasses were chilled for 24 h at –2 to 0 °C. Sides were ribbed between the 10^{th}-11^{th} rib and loin eye area was measured at the 10^{th} - 11^{th} rib interface to the nearest tenth of a square cm by using a plastic grid. Fat depth measurements were made to the nearest 0.25 of a centimeter at the first, tenth (fat over the loin eye, ¾ of the length of the eye from the bone side) and last ribs and the last lumbar by using a steel ruler.

Total loin dissection was done and primal to wholesale-ready cut measurements were taken. The primal loin from the right side of each carcass was dissected into component parts (lean, subcutaneous fat, intermuscular fat, bone, skin, and trim), and weights of each were recorded in kilograms. Each primal cut from the left side of the carcass (ham, loin, picnic, and butt) was then trimmed to .64 cm external fat wholesale-ready product. Bellies were skinned, squared, and spareribs removed in preparation for bacon production. Weights of

component parts (initial, trim, finished) were measured in kilograms. Belly hardness (firmness) was determined by suspending the longitudinal midpoint of the belly across a stainless steel rod and measuring the distance in cm between the ham and shoulder end, both lean side up and lean side down.

Lipid Analysis

Lipids in the lean tissue and subcutaneous fat samples were extracted by using the modified Folch method (1957), with chloroform/methanol (2:1, vol/vol). Lipids extracted from tissue samples were methylated (sodium methoxide) as follows: 2 g sample was dissolved in hexane (1 ml) in a test tube with a Teflon-lined screw cap, 0.5 M sodium methoxide in anhydrous methanol (2 ml) added, the solution maintained at 50 °C for 10 min, and glacial acetic acid (0.1 ml) added followed by deionized water (5 ml). The fatty acid methyl esters (FAME) were extracted into hexane (2 x 3 ml) and dried over anhydrous sodium sulfate. All FAME were analyzed using a gas chromatograph (HP 6890, autosampler 6890; Hewlett-Packard Co., Avondale, PA) equipped with an HP 19091J-413 column (30 m, 320 μm i.d., 0.25 μm film thickness: Hewlett-Packard Co., Avondale, PA) and operated at 180 °C for 0.5 min, (temperature programmed 2 °C / min to 230 °C and held for 4.50 min). The injector and flame-ionization detector temperatures were both set at 300 °C. FAME were identified by comparison of their retention times with an authentic standard (UC-59 MX, Nu-Check-Prep, Elysian, MN), and were verified with mass spectophotometry. Fat extractions were run three times and average CLA results as a percentage of total fatty acid is reported.

Statistical Analysis

Results were analyzed with the GLM procedures of SAS (1988) with the pig as the experimental unit. One pig was removed from the test due to lameness, therefore n = 39. General linear models included main effects for treatment and replication. Treatment effects were partitioned into linear and quadratic responses by orthogonal contrasts. Slaughter weight (114 kg) was included as a covariant for all analysis.

Results and Discussion

Pig performance (Table 3) as measured by average daily gain (ADG) improved linearly ($P < .01$) as concentration of CLA in the diet increased. The greatest difference was at the 1.0% level compared with the controls and these results suggested that even greater amounts of CLA in the diet might yield even greater improvements in ADG. ADG for pigs from all CLA diets was slightly suppressed (although not significantly) during the first two weeks of the study. After week two, however, ADG for CLA fed pigs tended to be greater (although not significantly) than for controls when measured at two week intervals. Pigs initially required adjustment time to any addition of CLA in their diet. Results on ADG reported in other studies have varied. Park et al. (1997) found no difference in ADG for male mice, but there was a slight (but not significant) decrease in ADG for females. In pigs, Dugan et al. (1997) found no differences in ADG between pigs fed CLA and pigs fed sunflower oil. Dunshae et al. (1998) described a trend toward increased ADG, whereas Cook et al. (1999) described a significant decrease in ADG from days 0 - 49 for CLA fed pigs.

There was also a slight suppression of feed intake for CLA fed pigs during the first two weeks. This supports a slight (but not significant) decrease in average daily gain seen

during the same period. From week two on, there was no difference in feed intake ($P = .85$). Increased gain/feed ($P < .01$) resulted from improved ADG without an increase in average daily feed intake (ADFI). No difference in feed intake was noted in rats by Chin et al. (1994), however; Park et al. (1997) demonstrated that intake was reduced during the first 15 days of a study with mice. Dugan et al. (1997) reported decreased feed intake from 60 - 105 kg live weight and Cook et al. (1999) reported decreased feed intake from 0 - 49 days for pigs.

In Table 4, data indicate tenth rib fat depth from CLA treated pork carcasses was less than tenth rib fat depth of controls ($P < .05$) in a quadratic relationship. No other fat depth measurements (first rib, last rib, last lumbar) exhibited a significant treatment difference at any level of CLA supplementation. Although not significant, first rib backfat tended to decrease linearly ($P = .07$) as demonstrated by a –3.91% difference at .12 % CLA addition and –15.65% at 1.0% CLA addition. Last rib and last lumbar fat depth results indicated no differences between treatments and controls.

Ultrasound backfat results over time demonstrated a quadratic effect of treatment. Fat depth decreased at approximately 91 kg of live weight for .12% treatment compared with the control. This difference ($P < .001$) was maintained at 114 kg for .12 and .25% CLA treatments as shown in Figure 1. Tenth rib fat depth results of carcasses confirmed tenth rib ultrasound measurement results of live animals. Cook et al. (1999) reported ultrasound backfat differences of 26% by the end of the feeding period for pigs fed .95% CLA and actual 10^{th} rib backfat at slaughter was 24.2% less than controls. Our ultrasound LEA results (Figure 2) also indicated an increase in LEA for 1.0% CLA treatments at approximately 91 kg compared with controls. This difference was maintained throughout the remainder of the

feeding period for 1.0% CLA treatments. Ultrasound loin eye area (LEA) results showed a linear effect of treatment with increased concentration of CLA in the diet. This result was not supported, however, by carcass LEA measurements, which found no statistical difference between controls and treatments (Table 4). Cook et al. (1999) also reported no significant differences between controls and treated pigs for measured loin eye area.

Initial loin weight (Table 5) demonstrated a quadratic effect of treatment ($P < .05$) with controls and 1.0% CLA treatments having higher initial weights than .12 and .25% CLA treatments. Less intermuscular fat was found at .12% CLA than in controls or .50 and 1.0% CLA treatments. Although there was a quadratic effect of treatment ($P < .05$) for subcutaneous fat there were no individual between treatment differences. There was a linear trend ($P = .08$) for increased finished loin weight. In general, this loin dissection data suggests that .12 and .25% CLA treatments were lighter initially, however, less fat (both intermuscular and subcutaneous) made their finished loin weight heavier than the controls. Dugan et al . (1997) also reported increased initial loin weight and decreased subcutaneous fat and intermuscular fat in pigs fed .50% CLA. Cook et al. (1999) dissected the loin section between the 6^{th} and 10^{th} ribs only and determined that the amount of lean increased ($P < .001$) by 10% in CLA-60 fed pigs.

An increase in bone weight was observed ($P < .05$) with .50 and 1.0% CLA treatments having more bone than did the controls, .12 and .25% CLA treatments. Increased cortical bone formation was demonstrated in chicks fed butter (a high source of CLA) by Watkins et al. (1997). Li and Watkins (1998) determined that CLA (1.0%) lowered *ex vivo* PGE_2 production in bone organ cultures suggesting that CLA has the potential to positively influence bone formation and resorption.

Our loin dissection data demonstrated that CLA produced a quadratic effect of treatment for both less intermuscular fat (.12% vs control, .50 and 1.0% CLA) and subcutaneous fat and a linear increase for bone, although finished loin weight only tended to increase. No differences were indicated between controls and treatments for skin ($P = .11$) and trim ($P = .13$); therefore, data are not presented in tabular form. Our loin dissection data showed that levels up to .50% CLA positively effected loin composition; consequently, there seemed to be a limiting effect in place by 1.0%.

Within individual wholesale-ready cuts, initial (primal) cut weight for ham increased linearly ($P < .05$), as did ham trim ($P < .05$) with the 1.0% CLA treatment results being different from controls. No differences were observed in finished (wholesale) ham weight (Table 6). Initial loin weight and finished loin weight were not different; however, loin trim was quadratic ($P < .05$) with .25% and .50% CLA treatments having less trim than did the controls. Initial picnic weight ($P < .05$) and picnic trim weight ($P < .01$) increased linearly with 1.0% CLA treated pigs different than those from the control. Combined, these produced no difference in finished picnic weight. Although no differences in either initial butt weight or butt trim weight were observed there was a linear increase in finished butt weight ($P < .05$) with the 1.0% CLA treatment being different from those of the controls. Initial belly weight ($P <.001$) and finished belly weight ($P < .001$) increased linearly with 1.0% CLA different from the controls. Belly trim exhibited a quadratic effect of treatment ($P < .001$) with .12% and .25% CLA having more trim than did those of the controls, .50% and 1.0% CLA treatments.

The most obvious difference observed during the primal to wholesale-ready part of the experiment was the hardness (firmness) of the belly. When belly firmness was measured

lean side up (Figure 3), a linear effect of treatment ($P < .001$) difference was observed with increasing amounts of CLA, and firmer bellies were found at .50 and 1.0% . A similar linear effect of treatment shown in Figure 4, was observed ($P < .05$) when bellies were measured lean side down. Firmer bellies provide a potential for improvement in sliceability and increase in yield of bacon. Cook et al. (1999) using the same measurement, also demonstrated an increase in belly firmness. This observation of increased belly firmness could be explained by an increase in saturated fatty acids. It is possible that CLA affects the saturate / monounsaturate ratio by inhibiting Δ-desaturase activity as suggested by Lee et al., (1995).

Results of CLA as a percentage of total lipids shown in Table 7 indicated that CLA was incorporated into both subcutaneous fat and lean tissue in increasing concentrations with increasing amounts of CLA in the diet. The results of the effect of CLA on fatty acid composition of subcutaneous fat and lean tissue are shown in Table 7. Lean tissue of pigs fed CLA had increased ($P < .01$) myristate (14:0) and ($P < .01$) palmitate (16:0) and decreased ($P < .01$) oleate (18:1) and ($P < .01$) arachadonate (20:4). Increased 14:0 and 16:0 and decreased 18:1 in lean tissue indicated a shift to more saturated fat. Subcutaneous fat of pigs fed CLA had increased 14:0 ($P < .01$), 18:1 ($P < .01$), and 18:2 ($P < .01$) and decreased 16:0 ($P < .01$). This shift towards saturated fat in lean tissue supports the incorporation of dietary CLA into tissues previously demonstrated by Chin et al. (1994), Park et al. (1997), Lee et al. (1996) and Sugano et al. (1997).). It has been demonstrated by Ha et al. (1990) and Yi-Chia Huang et al. (1994) that CLA is incorporated into phospholipids. Inclusion of CLA isomers into liver, heart, inner backfat, and omental fat of pigs was recently reported by Kramer et al. (1998). Cook et al. (1999) and Kramer et al. (1998) both reported that CLA

was incorporated into tissues in a dose dependant relationship. Banni et al. (1996) and Sebedio et al. (1997) suggested that the metabolites of CLA may be elongated and desaturated thus competing with other compounds to directly affect fatty acid composition. Turek et al. (1998) indicated that CLA fed to rats increased total spleen monounsaturated fatty acids but also increased the concentration of (n-3) polyunsaturated fatty acids.

Dietary supplementation of conjugated linoleic acid improved average daily gain and gain/feed in pigs fed from 26 – 114 kg. This improvement directly reduced the amount of feed required to finish pigs. Carcass composition was also improved with reductions in 10^{th} rib backfat and intermuscular and subcutaneous fat reductions from loin dissection. Unlike the linear effect of CLA on performance, carcass fat measurements were decreased quadratically. This relationship suggests that the amount of fat is reduced the most at dietary CLA levels of .50% or less. Belly firmness increased dramatically by addition of CLA to the diet. This increase is likely due to the incorporation of CLA isomers into the fatty acid profile of pork tissues or the change in the saturated / monounsatured fat ratio. The change in fatty acid profile is due to a shift toward higher concentrations of saturated fatty acids and lower concentrations of unsaturated fatty acids. Further study to define the most appropriate dose required to optimize pig performance, economy of gain, and body composition must be accomplished to assist in the practical application of CLA in pig diets. Also, identifying the active isomer(s), mechanism of biological activity and impact on human health are further areas of creative investigation.

IMPLICATIONS

The increase in average daily gain without an increase in average daily feed intake demonstrated by CLA supplementation could save feed in the growing-finishing phase for pigs in the United States. Decreased fat deposition and increased firmness of bellies should produce less waste from trim and improved bacon slicability and yield. The incorporation of CLA into lean tissues should positively impact the health of consumers. Further study to define the most appropriate dose requirement to optimize pig performance and body composition must take place. Also, identifying the active isomer(s) and mechanism of biological activity must be defined.

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Table 1. Fatty acid composition of the conjugated linoleic acid source[a] used for dietary supplementation

Fatty acid		g / 100g oil
16:0		4.6
18:0		3.4
18:1		23.8
18:2		7.7
Total CLA isomers[b]		
	c9, tll and t9, cll	12.3
	t10, c12	14.0
	t9, t11 and t10, t12	5.2
	c8, t10 and t8, c10	8.3
	c9, c11	1.3
	c11, t13 and t11, c13	12.2
	c8, c10	.5
	c10, c12	2.1
	c11, c13	.4
	other	4.2

[a] Synthesized by Pharma Nutrients, Oak Brook, IL

[b] c = cis, t = trans

Table 2. Composition of the basal experimental diet fed to growing-finishing pigs

Ingredient	%
Corn, ground[a]	70.13
Soybean meal, dehulled	27.13
Dicalcium phosphate	1.28
Calcium carbonate	.86
Salt	.25
Vitamin premix[b]	.20
Mineral premix[c]	.05
Antimicrobial[d]	.10

[a]CLA source (65% CLA) was substituted for corn in the diet. Diets containing .12% CLA included 69.93% corn, .25% CLA included 69.71% corn, .50% CLA included 69.30% corn, and 1.0% CLA included 68.46 % corn.
[b]Contributed the following per pound of diet: 1,010 IU of vitamin A, 250IU of vitamin D_3, 5 µg of vitamin B_{12}, 1.5 mg of riboflavin, 4.0 mg of d-pantothenic acid, and 7.5 mg of niacin.
[c] Contributed the following per pound of diet: 45 mg of Zn, 22.7 g of Fe, 2.5 mg of Cu, 12.5 mg of Mn, and .34 mg of I.
[d] Contributed 40 mg tylosin per pound of diet.

Table 3. Growth performance of growing-finishing pigs fed diets containing varying amounts of conjugated linoleic acid

	% Dietary CLA Added					
ITEM	0	.12	.25	.50	1.0	SEM
ADG, kg	.942[b]	.930[b]	.953[b]	.974[ab]	1.019[a]	.183
ADFI, kg	2.683	2.538	2.556	2.633	2.634	.052
Gain/feed	.352[b]	.367[ab]	.373[ab]	.370[ab]	.384[a]	.008

[a,b,c] Values with different superscripts within a row indicate linear treatment effects ($P < .05$)

Table 4. Dietary conjugated linoleic acid effects on fat depth and loin eye area of live animals (ultrasound) and carcasses

	% Dietary CLA Added					
ITEM	0	.12	.25	.50	1.0	SEM
First rib fat depth, cm	4.08	3.93	3.53	3.78	3.45	.22
Last rib fat depth, cm	2.61	2.85	2.49	2.51	2.69	.22
Last lumbar fat depth, cm	2.41	2.42	2.38	2.37	2.59	.19
Tenth rib fat depth, cm	2.86^{a}	2.34^{b}	2.34^{b}	2.61^{ab}	2.57^{ab}	.16
Loin eye area, cm^2	41.22^{ab}	43.85^{a}	42.03^{ab}	40.08^{ab}	39.28^{b}	1.35
Ultrasound backfat, cm	2.44^{a}	2.15^{b}	2.16^{b}	2.28^{ab}	2.37^{ab}	.10
Ultrasound loin eye area, cm^2	42.6^{a}	43.7^{a}	45.6^{ab}	44.9^{ab}	47.2^{b}	1.24

[a,b,c] Values with different superscripts within a row indicate quadratic treatment effects ($P < .05$)

Table 5. Loin dissection component weights, means, standard error, and P-values from growing-finishing pigs fed varying amounts of conjugated linoleic acid

Component	% Dietary CLA Added	Means (kg)	SEM	P
Initial Loin, kg	0	11.26[ab]	.31	.04[z]
	.12	10.89[a]	.31	
	.25	10.68[a]	.31	
	.50	11.54[ab]	.34	
	1.0	11.89[b]	.31	
Intermuscular fat, kg	0	.25[d]	.02	.005[z]
	.12	.15[bc]	.02	
	.25	.20[bd]	.02	
	.50	.22[d]	.03	
	1.0	.27[ad]	.02	
Subcutaneous fat, kg	0	2.86	.19	.03[z]
	.12	2.37	.19	
	.25	2.39	.19	
	.50	2.62	.20	
	1.0	2.84	.19	
Bone, kg	0	1.56[ab]	.06	.03[y]
	.12	1.56[ab]	.06	
	.25	1.46[b]	.06	
	.50	1.73[a]	.07	
	1.0	1.71[a]	.06	
Finished loin, kg	0.0	3.88	.13	.08[y]
	.12	4.00	.13	
	.25	4.04	.13	
	.50	4.07	.14	
	1.0	4.24	.13	

[a,b,c,d] Values with different superscripts within a column and category indicate treatment effects ($P < .05$)

[y] Linear effects of treatment

[z] Quadratic effects of treatment

Table 6. Weights, means, standard error and P-values for primal cuts trimmed to wholesale ready product from growing-finishing pigs fed varying amounts of conjugated linoleic acid

Primal wt, kg					Wholesale wt, kg					Trim wt, kg				
	%CLA	Mean	SE	P		%CLA	Mean	SE	P		%CLA	Mean	SE	P
Initial Ham	0	10.08[a]	.28	.02	Finished Ham	0	7.56	.25	.07	Ham Trim	0	2.48[ac]	.08	.04
	.12	10.18[ab]	.28			.12	7.73	.25			.12	2.42[a]	.08	
	.25	10.33[ab]	.28			.25	7.88	.25			.25	2.40[a]	.08	
	.50	10.55[ab]	.30			.50	7.99	.28			.50	2.52[c]	.08	
	1.0	10.94[b]	.28			1.0	8.20	.25			1.0	2.69[bc]	.08	
Initial Loin	0	11.71	.38	.53	Finished Loin	0	8.06	.29	.08	Loin Trim	0	3.66[a]	.22	.05[z]
	.12	11.57	.38			.12	8.31	.29			.12	3.25[ab]	.22	
	.25	11.64	.38			.25	8.64	.29			.25	2.98[b]	.22	
	.50	11.57	.41			.50	8.57	.32			.50	2.99[b]	.24	
	1.0	12.09	.38			1.0	8.77	.29			1.0	3.31[ab]	.22	
Initial Picnic	0	4.65[a]	.14	.03	Finished Picnic	0	2.64	.12	.30	Picnic Trim	0	2.00[a]	.08	.01
	.12	4.81[ab]	.14			.12	2.79	.12			.12	2.04[a]	.08	
	.25	4.76[ab]	.14			.25	2.82	.12			.25	1.93[a]	.08	
	.50	4.94[ab]	.15			.50	2.78	.13			.50	2.13[ab]	.08	
	1.0	5.12[b]	.14			1.0	2.85	.12			1.0	2.28[b]	.08	
Initial Butt	0	4.49	.15	.23	Finished Butt	0	2.46[ab]	.10	.05	Butt Trim	0	1.97	.10	.89
	.12	4.53	.15			.12	2.34[a]	.10			.12	2.15	.10	
	.25	4.54	.15			.25	2.44[ab]	.10			.25	2.25	.10	
	.50	4.49	.16			.50	2.52[ab]	.11			.50	1.98	.11	
	1.0	4.79	.15			1.0	2.69[b]	.10			1.0	2.08	.10	
Initial Belly	0	7.96[a]	.24	.004	Finished Belly	0	5.20[a]	.20	.006	Belly Trim	0	2.55[a]	.16	.003[z]
	.12	8.02[a]	.24			.12	4.90[a]	.20			.12	3.03[ab]	.16	
	.25	8.53[ab]	.24			.25	5.16[a]	.20			.25	3.37[b]	.16	
	.50	8.36[ab]	.26			.50	5.39[ab]	.22			.50	2.93[a]	.18	
	1.0	8.99[b]	.24			1.0	5.92[b]	.20			1.0	2.77[a]	.16	

[a,b,c,d] Values with different superscripts within a column category indicate treatment effects ($P < .05$)

[z] Indicates quadratic effects of treatment

Table 7. Fatty acid profile of subcutaneous fat and lean tissue of pigs fed varying amounts of CLA[a]

Component	% Dietary CLA	Fatty acid, Relative %										
		14:0	16:0	16:1	18:0	18:1	18:2	20:4	c9,t11- & t11,c9[b] CLA	t10,c12 CLA	t9,t11 CLA	t10,t12 CLA
Subcutaneous Fat												
	0	1.76^{d}	30.63^{d}	1.93^{d}	12.87	25.80^{d}	16.71^{d}	.15	nd^{cd}	nd^{d}	nd^{d}	nd^{d}
	.12	1.84^{e}	27.12^{de}	2.32^{ef}	13.86	27.44^{e}	17.47^{d}	.17	.33^{e}	.19^{e}	nd^{d}	nd^{d}
	.25	1.79^{d}	31.63^{d}	2.50^{f}	12.29	29.42^{f}	19.42^{e}	.14	.81^{f}	.44^{f}	nd^{d}	nd^{d}
	.50	1.90^{e}	24.77^{e}	2.33^{ef}	13.73	30.97^{g}	20.24^{e}	.15	1.21^{g}	.99^{g}	.15^{e}	.26^{e}
	1.0	2.12^{e}	20.41^{e}	1.96^{de}	12.34	31.51^{g}	20.24^{e}	.16	2.16^{h}	1.87^{h}	.35^{f}	.47^{f}
Pooled SEM		.06	1.43	.14	.81	.50	.50	.01	.08	.46	.16	.12
Lean Tissue												
	0	1.00^{d}	21.81^{d}	2.72^{d}	12.40	35.85^{df}	11.99^{d}	1.45^{d}	.03^{d}	nd^{d}	nd^{d}	nd^{d}
	.12	1.04^{de}	22.75^{de}	3.10^{de}	11.69	37.17^{d}	12.03^{d}	1.51^{d}	.08^{d}	.04^{d}	nd^{de}	nd^{d}
	.25	1.16^{ef}	24.10^{ef}	3.31^{e}	11.15	35.67^{df}	12.88^{de}	1.29^{de}	.19^{e}	.14^{e}	.03^{ef}	.03de
	.50	1.19^{f}	24.94^{f}	3.34^{e}	11.89	33.96^{de}	13.87^{e}	1.06^{f}	.26^{f}	.19^{e}	.04^{f}	.04^{ef}
	1.0	1.34^{g}	26.51^{g}	4.43^{f}	11.44	32.11^{e}	13.01^{de}	1.10^{ef}	.37^{g}	.32^{f}	.05^{f}	.06^{f}
Pooled SEM		.04	.05	.17	1.69	1.12	.44	.08	.02	.02	.007	.01

[a] Values are arithmetic means for eight observations

[b] c = cis, t = trans

[c] nd (not detected) levels were below the detection level of .04%

[d,e,f,g,h] Values with different superscripts within a column category indicate linear effects of treatment ($P < .01$)

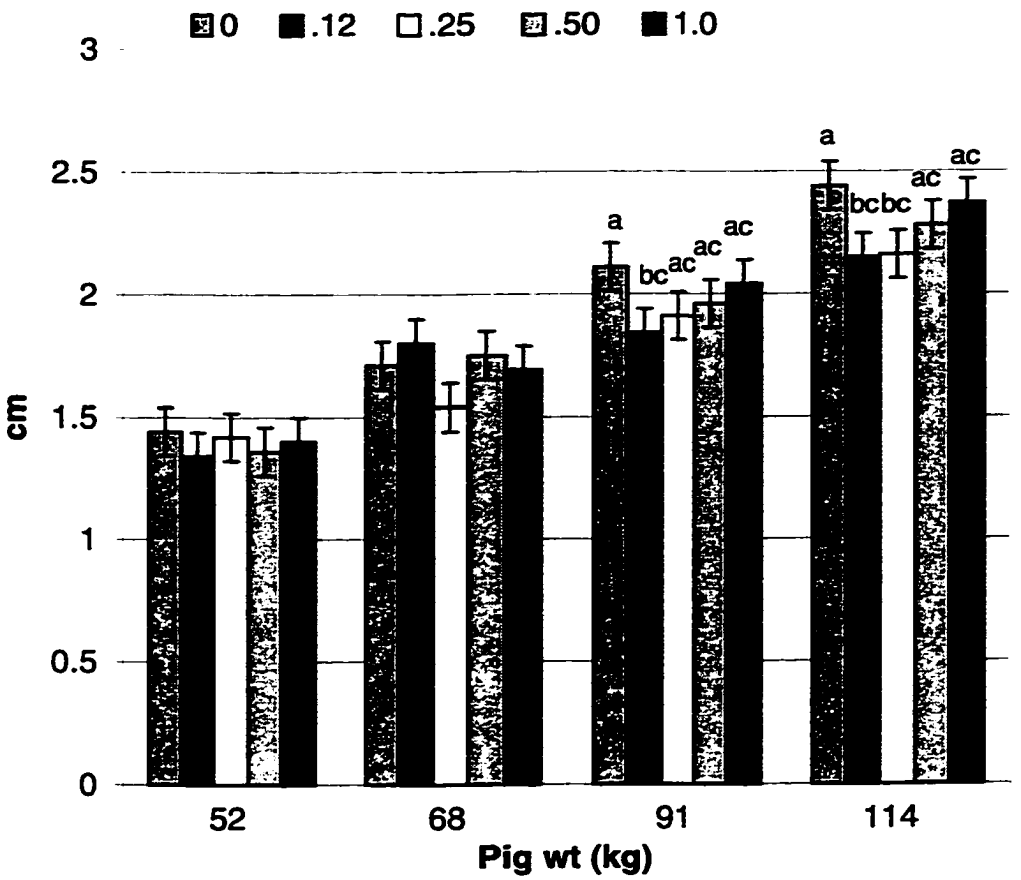
Figure Legends

Figure 1. Effect of dietary CLA (0 = control, .12 = .12% CLA, .25 = .25% CLA, .50 = .50% CLA, 1.0 = 1.0% CLA) on 10^{th} rib fat depth (cm) of growing-finishing pigs measured by using ultrasound

Figure 2. Effect of dietary CLA (0 = control, .12 = .12% CLA, .25 = .25% CLA, .50 = .50% CLA, 1.0 = 1.0% CLA) on loin eye area (cm^2) of growing-finishing pigs measured by using ultrasound

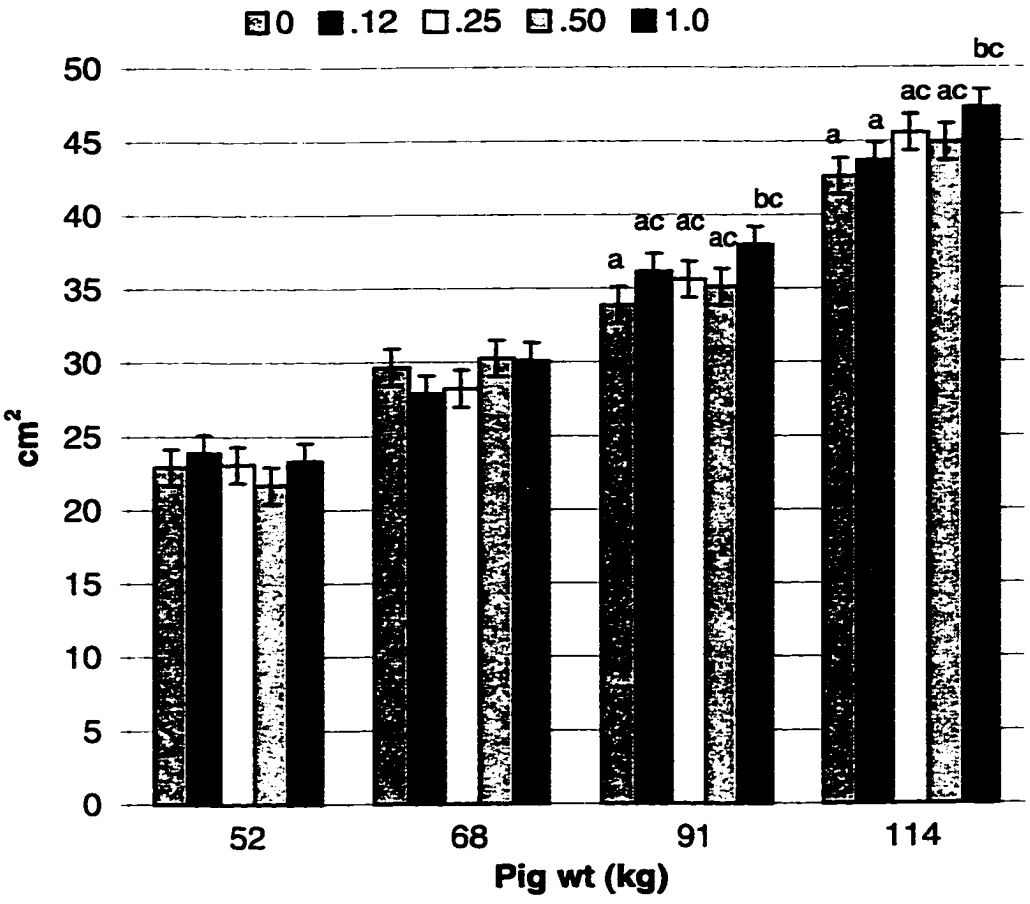
Figure 3. Belly firmness measured between the shoulder and ham ends (cm) lean side up as affected by dietary conjugated linoleic acid

Figure 4. Belly firmness measured between the shoulder and ham ends (cm) lean side down as affected by dietary conjugated linoleic down



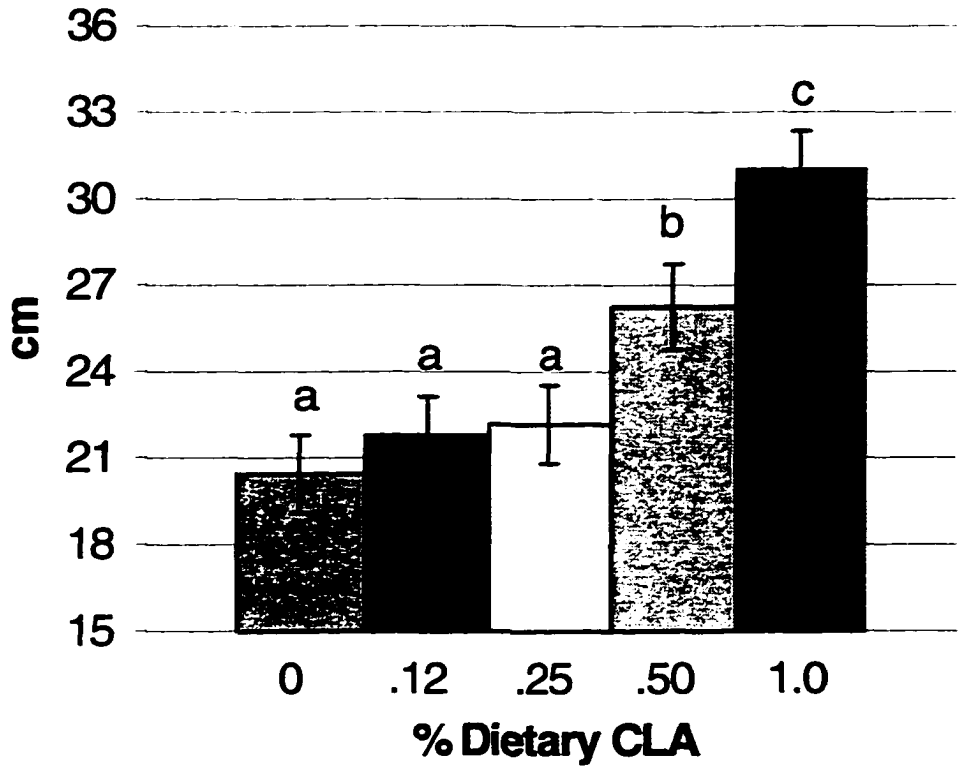
[a,b,c] Values with different superscripts within a weight category indicate linear treatment effects ($P < .05$)

Figure 1.



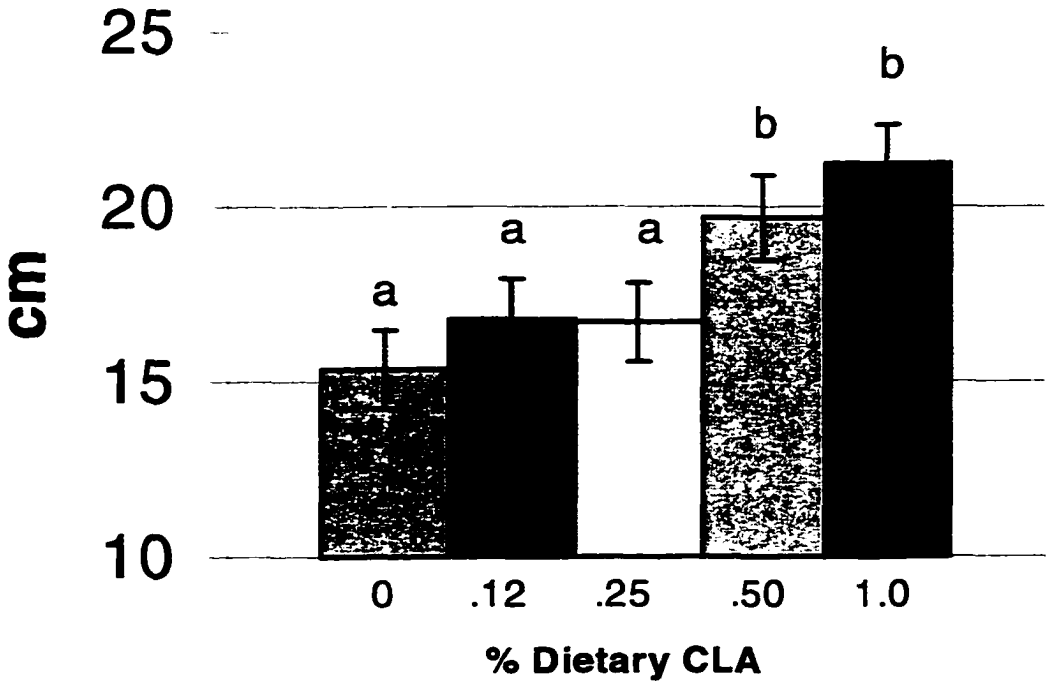
[a,b,c]Values with different superscripts within a weight category indicate linear treatment effects ($P < .05$)

Figure 2.



[a.b.c] Values with different superscripts indicate linear treatment effects ($P < .007$)

Figure 3.



[a,b] Values with different superscripts indicate linear treatment effects ($P < .05$)

Figure 4.

SENSORY AND PHYSICAL CHARACTERISTICS OF PORK FROM PIGS FED CONJUGATED LINOLEIC ACID SUPPLEMENTED DIETS

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ABSTRACT

Conjugated linoleic acid improves performance and body composition in lab animals and pigs; however, its effects on sensory and physical characteristics of pork have not been investigated. An experiment was conducted to determine the effects of feeding conjugated linoleic acid (CLA) on sensory characteristics of pork fresh loin chops. Eight replications of five littermate barrows (starting wt = 26.3 kg) were fed diets containing 0, .12, .25, .50, or 1.0% CLA by weight of the diet. Pigs were slaughtered at 116 kg live weight. Three studies to evaluate quality and sensory characteristics of fresh pork were conducted. In the first study of day one postmortem fresh loin chops, no significant differences were found between chops from CLA-fed and control pigs for 24 h pH, water-holding capacity and Warner-Bratzler shear force. Hunter color a* values increased linearly ($P < .05$) with the amount of CLA added in the diet. Subjective color, marbling, and firmness scores did not display any significant differences at the $10^{th} - 11^{th}$ rib interface. A sensory panel of day one postmortem loin chops indicated that control chops were more juicy (at the first bite) than were all of the treatments. The second study of fresh loin chops (0 or 1.0% CLA) held for 7, 14, 28, or 56 days of storage at 3 °C found no treatment differences for Hunter L*, a*, and b* values. Trained panelists were unable to determine differences between treatments for tenderness or juiciness at the different aging times. The third study was a preliminary study of 70/30 fresh pork patties from one replication only. This study suggests that L*, a*, and b* values were

effected by dietary CLA at day one postmortem. A sensory panel was unable to determine a difference from the control at d 1 of storage but tended to find a difference at d 3 and d 7 for .50 and 1.0% CLA. Dietary CLA had no significant impacts on sensory or quality characteristics of fresh pork.

Key words: Conjugated linoleic acid, pork loin chops

INTRODUCTION

Conjugated linoleic acid (CLA) consists of a group of positional and geometric isomers of linoleic acid. While linoleic acid has double bonds between the 9^{th} and 10^{th} carbons and the 12^{th} and 13^{th} carbons, CLA has double bonds at carbon atoms 10 and 12, or 9 and 11, with possible *cis* and *trans* isomers. It is believed that the c9, tll-CLA and / or t9, c11-CLA is (are) the most biologically active (Belury, 1995). When a mixture of CLA isomers was introduced the c9, t11- and t9, c11-CLA were preferentially incorporated into tissue lipids of the forestomach (Ha et al., 1990), liver, and mammary tumor (Ip et al., 1991). CLA has been shown to have anticarcinogenic, and antiatherogenic effects. CLA has been shown to prevent mammary cancer (Ip et al., 1991, Ip and Scimeca, 1997, and Ip et al., 1997), skin cancer (Ha et al.,1990, and Belury et al., 1996), colon and prostate cancer (Liew et al., 1995 and Cesano et al., 1998) and lung cancer (Schønberg and Krokan, 1995). Antiatherogenic effects of CLA have been reported in experiments with rabbits (Lee et al., 1994) and hamsters (Nicolosi et al., 1997). The anticarcinogenic and antiatherogenic activity of CLA was suggested to be involved in oxidative stress as an antioxidant (Ha et al. 1990 and Ip et al. 1994). Several studies have since been completed which cast doubt on the theory that CLA is an antioxidant. Studies by van den Berg et al. (1995) demonstrated that CLA was not

an efficient radical scavenger or metal scavenger when tested under metal-ion dependant oxidative stress. Chen et al. (1997) determined that CLA does not serve as an antioxidant in fats and oils. Taken together these studies suggest that CLA may not have a direct antioxidant activity.

One study (Cook et al., 1999) investigated the effects of CLA on fresh meat quality or sensory characteristics by subjective color scores and Minolta L* values. This study in pigs found that Minolta L* values tended to be lower at the 10^{th} – 11^{th} rib interface, indicating that CLA supplementation produced darker lean color. Cook et al. (1999), also found no differences in subjective firmness and marbling, however, subjective color score increased with additional CLA in the diet.

No studies have been reported relating the dietary effects of CLA supplementation on sensory panel evaluations and sensory attributes in any meat animal species. Therefore, this study was undertaken in an effort to examine the effects of dietary CLA supplementation on quality and sensory characteristics of fresh pork products.

MATERIALS AND METHODS

Animals and Diets

Eight replications of five littermate barrows (Yorkshire x Landrace x Hampshire x Duroc) with an average weight of 26.3 kg each, were allotted at random to individual pens. Within replication dietary treatments containing 0, .12, .25, .50, 1.0% CLA were assigned at random. Diets were reformulated at approximately 3 wk intervals (six formulations) to reduce crude protein and lysine throughout the feeding period. Pigs were harvested at an

average weight of 116 kg at the Iowa State University Meat Laboratory according to humane slaughter requirements.

Carcass characteristics

Post slaughter, the carcasses were chilled for 24 hours at –2 – 0 °C. Carcasses were ribbed at the 10^{th} – 11^{th} rib interface and color, firmness and marbling were scored subjectively using NPPC standards (1991). Samples of loin muscle tissue and subcutaneous fat (innermost layer) were taken for lipid extraction. Total lipid percentage was calculated by a modified Folch procedure (1957), which uses chloroform/methanol extraction (% total crude lipids = lipid wt x chloroform vol (ml) / 10 ml / sample wt x 100).

pH and Water Holding Capacity

Samples of loin tissue were taken at the 10^{th} rib for pH_{24} (pH at 24 h postmortem) and water holding capacity. pH_{24} was measured with a Fischer (Accumet 125) pH meter on a ten-gram homogenized sample (in 90 g distilled water) in duplicate. Water holding capacity was determined by using the Carver press method (Kauffman et al., 1986). In this method a .3 g sample was placed between two pieces of Whatman #1 filter paper and submitted to 3000 psi of pressure for 3 minutes. The two areas (total area, meat area) were outlined on the filter paper and measured with a planimeter. The higher the measured ratio (total area/meat area) the lower the relative water holding capacity. Samples were run in duplicate and average results are reported.

Hunter and Warner-Bratzler Shear – Loin Chops

Four 2.54 cm thick loin chops were cut from the left side of each carcass. Two chops were used for both Hunter color and Warner-Bratzler shear (WBS) analysis at day one postmortem. Two additional chops from pigs fed control and 1.0% CLA diets were vacuum

packaged at day one postmortem and held at 3 °C for 7, 14, 28 or 56 days and then frozen for subsequent Hunter color and sensory panel evaluation. Each loin chop was measured at 3 locations (dorsal, central and ventral) with a 1.27 cm appeture, D65 light source, and 10° observer for CIE (1976) color determinations using a Hunter LabScan. Hunter color measurements were made under vacuum. CIE, L*, a* and b* values were recorded at appropriate days postmortem. The chops were cooked to 70 °C internal temperature and allowed to cool for 24 hours to 2 °C for WBS analysis. Three 1.27 cm diameter cores were removed parallel to the muscle fiber direction from the central, medial and lateral portions of the chops. Cores were sheared through the center, perpendicular to the fiber direction. WBS force was measured with an Instron universal testing machine (model 4502) controlled by a model 4500 computer assist module (Instron, Canton, MA). Peak shear force values were recorded as kg / 1.27 cm diameter core.

Sensory Panel – Loin Chops

Two additional 2.54 cm chops from the left side of the carcass were cut, vacuum packaged and frozen at d 1 postmortem for subsequent sensory panel analysis. The two chops used for Hunter analysis of color shelf life described above were also used for sensory panel analysis. Chops were thawed in a refrigerator (3 °C) for 26 hours before cooking. Shelf life loin chops were weighed in the bag, removed from the bag and weighed again to determine drip loss. Two chops from each animal were weighed and then broiled in a conventional household oven with the broiling rack positioned 15 cm under the heating element. Temperature was monitored by inserting a probe (Omega Engineering Inc., Stamford, CT) into the geometric center of each chop. The transition joint probes were stainless steel with a sheath diameter of .158 cm and a length of 10.16 cm. They were

connected to chromel-alumel lead wires, which were attached to a DP82 Digital Process Indicator (Omega Engineering Inc., Stamford, CT). The chops were turned at 40 °C, removed from the oven at 68 °C, and allowed to stand until the internal temperature registered 71 °C. The chops were weighed (to determine cook loss %) and cut into 1.27 cm cubes. The pieces from the chops from one animal were mixed and the two randomly selected cubes were placed in a preheated glass dish, which was then covered. Dishes were marked with a three digit random code. Samples were served warm to panelists.

Ten panelists were trained to evaluate initial juiciness, tenderness, sustained juiciness, overall tenderness, and pork flavor. Initial juiciness was defined as the amount of juice released on the first bite. Tenderness was defined as the force to bite through the sample with the molars. Sustained juiciness was defined as impression of juiciness throughout chewing. Overall tenderness was defined as force to disintegrate sample to a state ready to swallow. Pork flavor was defined as the flavor of the broiled lean from pork loin. During training, panelists were presented with meat samples selected or prepared to exhibit varying degrees of these characteristics. An eight point category scale (ex. 8 = extremely juicy, 1 = extremely dry) was used to indicate the degree of each attribute (AMSA, 1995). Five treatments were presented in each of eight sessions (except for session eight in which treatment five was missing) for the day one postmortem panels. Each panelist evaluated all of the treatments each session. For a particular session of the shelf life study (control and 1.0%), samples were randomly chosen from each of the treatments. Each day combination was presented in each of six sessions. For a particular session, samples were randomly chosen from each of the treatments and time combinations. Testing was done in partitioned booths under red light.

Hunter Color - Pork Patties

A preliminary study using picnic shoulders from two repetitions were trimmed to .32 cm exterior fat and ground together into 70/30 (70% lean / 30% fat) fresh pork patties. Two patties per dietary treatment per repetition combination were placed in a styrofoam tray, packaged in fresh overwrap and held at 3 °C for 1, 3, 7 or 14 days prior to freezing. Patties were frozen in original packages inside plastic lined boxes. Frozen pork patties, in their original packages, were thawed in a refrigerator (3 °C) for 18 hours and color readings were taken (LabScan, Hunter Associates Laboratory Inc., Reston, VA). One center reading and four side readings (L*, a*, b*) were taken by using the 1.27 cm sample port insert, under D65 illuminant and 10° observer.

Sensory Panel – Pork Patties

The raw patties were weighed and then cooked in preheated household electric skillets set at 163 °C. The patties were cooked for two minutes, turned, and cooked an additional two minutes. After four minutes, the patties were turned as frequently as needed to prevent sticking and burning. The end point temperature of 71 °C, taken in the center of the patty, was monitored by using Teflon coated chromal-alumel lead wires attached to a DP82 Digital Process Indicator (Omega Engineering, Stamford, CT). After weighing, each patty was cut into 8 wedges and the wedges from the two patties in each treatment group were mixed. Panelists were served warm samples (37 °C ± 3°) in covered styrofoam containers labeled with a random three-digit code.

Six samples were presented to eight untrained panelists. The sample with 0% CLA served as the labeled control each time. Panelists were asked to evaluate how different the

remaining five samples (blind control plus four levels of CLA) were from the labeled control. Three independent sessions were conducted and each session consisted of a set of samples from one of the three storage times. Day 14 patties were excluded because control patties were inedible due to rancidiy. Testing order was randomized among panelists. Testing was done by using a computerized sensory software system (COMPUSENSE five, Compusense, Inc., Guelph, Canada) and in partitioned booths under red light.

Statistical Analysis

Results were analyzed with the GLM procedures of SAS (1988) with the pig as the experimental unit. One pig had to be removed from the test due to lameness, therefore n = 39. General linear models included main effects for treatment and replication. Treatment effects were partitioned into linear and quadratic responses by orthogonal contrasts.

RESULTS AND DISCUSSION

The results in Figure 1 show subjective color, firmness, marbling and % lipid values which indicated no significant differences between treatments and controls. No significant effects of treatment for firmness were indicated although there was a trend ($P = .07$) for decreased firmness of CLA treatments compared to controls. The results of objective measures of pork quality at 24 h postmortem, shown in Table 1 indicated no differences in pH_{24}, WHC and WBS values. Hunter a* values increased ($P < .05$) as the amount of CLA added to the diet increased (Table 2), however, Hunter L* and b* values were not different. The increase in a* demonstrated that treated loin chops were more red than the controls. Cook et al., (1999) demonstrated an increase in subjective color scores and a decreased

Minolta L* value for CLA treated pigs. Their results indicate that the darker color observed subjectively was due to lower Minolta L* values.

Trained sensory panel results from d 1 postmortem loin chops indicated only that controls were more juicy initially ($P < .05$) than .12% chops (Table 3). No differences were noted for sustained juiciness, tenderness, overall tenderness or pork flavor by trained panelists. Percentage cook loss (Figure 2) from d 1 postmortem loin chops showed no differences between treatments and controls. The results of the d 1 postmortem study demonstrated that for d 1 postmortem loin chops the only pork quality or sensory characteristic with a substantive difference was color. In our study this difference was in Hunter a* (redness) values while Cook et al., (1999) suggests this difference was due to Minolta L* (lightness) values. Although there is agreement that d 1 color is enhanced by dietary CLA supplementation, exactly what factors are involved is unknown.

In the second study comparing only chops from pigs fed either 0 or 1.0% CLA, Hunter values for loin chops held for 7, 14, 28, or 56 days of fresh storage did not display any differences in L* ($P = .16$), a* ($P = .16$), and b* ($P = .96$). In addition, no differences in drip loss ($P = .48$) or cook loss ($P = .78$) were noted (Figure 3). A trained sensory panel found no differences between controls or 1.0% loin chops from pigs fed CLA in any of the categories measured; initial juiciness, sustained juiciness, tenderness, overall tenderness or pork flavor. There were, however, highly significant interactions between days within treatment as shown in Table 5. Control chops had less initial juiciness, sustained juiciness, tenderness, and overall tenderness at d 7 than at d 14, d 28, and d 56. Loin chops from 1.0% CLA fed pigs had lower initial juiciness and sustained juiciness at d 7 and d 56 than at d 14 and d 28. Both tenderness and overall tenderness was greater at d 28 and d 56 that at d 7 and

d 14 in loin chops from 1.0% CLA fed pigs compared to controls. Pork flavor indicated a treatment x day interaction ($P < .03$) with controls at d 14 having more pork flavor than controls at d 7 and d 28 and 1.0% CLA treated at d 7, d 14, and d 56. No other differences in pork flavor were observed. The results of loin chops held 7, 14, 28 or 56 days demonstrated no distinctive differences between controls and 1.0% loin chops from CLA fed pigs. Both controls and 1.0% CLA supplemented chops did exhibit increased juiciness (initial and sustained) and tenderness (initial and overall) beginning with d 14 of fresh storage within treatments.

The preliminary study using 70/30 fresh pork patties was designed to evaluate patties at 1, 3, 7 and 14 days of storage in fresh overwrap packaging. Although product was maintained for 14 days the control patties had a high degree of rancidity by sensory determination (color and odor) and were therefore not included in evaluations. Untrained panelists seemed to differentiate between controls and .50 and 1.0% CLA supplemented patties (Figure 4) at d 3 and d 7. Cook losses seemed to be similar across treatments (Figure 5). Hunter L*, a*, and b* results are shown in Figures 6a, 6b, and 6c respectively. L* values suggest that at d 1 the controls were lighter than the patties from .12, .25 and .50% dietary CLA supplementation. However, at d 3 and d 7 only the .50% patty was darker than the control. The a* values suggest that the .50% patty from CLA fed pigs were the most red at d 1 and d 3 of storage and that all CLA patties (.12, .25, .50 and 1.0%) were more red than controls at d 7 of storage. Hunter b* values did not demonstrate a clear difference between controls and supplemented patties.

The apparent ability of CLA to maintain both L* and a* values in fresh pork patties may be due to a shift in fatty acid composition or decreased oxidation. Thiel-Cooper et al.,

(1999) found that CLA increased the concentrations of 14:0 and 16:0 and decreased 18:1 concentrations in pork lean tissue. Li and Watkins, (1998) found that CLA decreased the concentration of 16:1n-7, 18:1, total monounsaturates, and n-6, but increased concentrations of 22:5n-3, 22:6n-3, total n-3, and saturates in most tissue analyzed. These results suggest that CLA caused a shift in fatty acid composition from unsaturated to saturated fatty acids. It is possible that CLA affects the saturate / monounsaturate ratio by inhibiting Δ9-desaturase activity as suggested by Lee et al., (1995). Banni et al., (1995) demonstrated that the metabolites of CLA are elongated and desaturated which may also alter fatty acid composition.

CLA has been demonstrated to have anticarcinogenic (Ha et al. 1990, Ip et al. 1994, and Liew et al. 1995) and antiatherogenic (Lee et al. 1995 and Nicolosi et al. 1997) activity in animal models. This activity was suggested to be involved in oxidative stress as an antioxidant (Ha et al. 1990 and Ip et al. 1994). Several studies have since been completed which cast doubt on the theory that CLA is an antioxidant. Studies by van den Berg et al. (1995) demonstrated that CLA was not an efficient radical scavenger or metal scavenger when tested under metal-ion dependant oxidative stress. Chen et al. (1997) determined that CLA does not serve as an antioxidant in fats and oils. Taken together these studies suggest that CLA may not have a direct antioxidant activity.

Oxidative changes have been found to be much greater in tissue fractions containing phospholipids such as lean muscle tissue. Unsaturated fatty acids oxidize more quickly than do saturated fatty acids and unsaturated fatty acids are found in greater percentages in the phospholipid fraction. Marked color, flavor and odor changes occur in phospholipids exposed to air. Darkening of phospholipids accompanies the development of rancidity

(Dugan, 1987). Thus, the stability of color in fresh pork patties is most likely due to the rate of change in the saturate / monounsaturate ratio making the product less susceptible to lipid oxidation.

IMPLICATIONS

Conjugated linoleic acid (CLA) increased Hunter a* values in pork loin chops at d 1 postmortem. This increased redness without other sensory or quality changes suggests that CLA may have effects on initial pork color. The initial increase in pork color was not maintained in loin chops held for 7, 14, 28, or 56 days at 3 °C of fresh storage. Panelists were unable to discern differences between chops from CLA fed pigs and controls at d 1 with the exception of initial juiciness. Although both controls and chops from 1.0% CLA fed pigs had increased juiciness (initial and sustained) and tenderness (initial and overall) at d 14 and later of fresh storage no between treatment differences were noted. Preliminary fresh pork patty results suggest that dietary CLA supplementation maintains patty color through d 7 of fresh overwrap storage. No differences in percentage cook loss were observed in any of the studies. CLA supplementation of growing-finishing pig diets will maintain existing loin chop and fresh pork patty sensory and quality characteristics.

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Table 1. Mean values of pH, water holding capacity and Warner-Bratzler shear force on pork loin chops at one day postmortem from pigs fed differing amounts of CLA

	% Dietary CLA					
Component	0	.12	.25	.50	1.0	SEM
pH_{24}[a]	5.61	5.60	5.58	5.53	5.54	.50
WHC[b]	3.50	3.64	3.40	3.59	3.80	.26
WBS[c]	2.98	2.50	2.91	3.06	2.74	.22

[a] pH measured at 24 h postmortem
[b] Water holding capacity
[c] Warner-Bratzler shear force

Table 2. Mean values of Hunter L*, a*, and b* on pork loin chops at day one postmortem from pigs fed differing amounts of CLA[a]

% Dietary CLA	L*	a*	b*
0	53.40 ± 1.73	.80[cd] ± .57	11.33 ± .56
.12	52.03	.94[cd]	10.63
.25	54.43	1.60[bd]	11.43
.50	53.80	1.54[bd]	11.03
1.0	54.83	2.95[b]	11.52

[a] Values are arithmetic means ± SEM for eight observations
[b,c,d] Values with different superscripts within a column indicate linear treatment effects ($P < .05$)

Table 3. Mean values of sensory panel results for day one postmortem pork loin chops from pigs fed differing amounts of CLA[a]

% Dietary CLA	Initial Juiciness	Sustained Juiciness	Tenderness	Overall Tenderness	Pork Flavor
0	4.63[b] ± .21	4.58 ± .19	4.54 ± .21	4.86 ± .20	4.64 ± .18
.12	3.96[cd]	3.98	4.46	4.60	4.39
.25	4.19[d]	4.42	4.82	5.01	4.35
.50	4.18[d]	4.22	4.51	4.66	4.47
1.0	4.25[d]	4.30	4.80	5.05	4.56

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d] Values with different superscripts within a column indicate linear treatment effects ($P < .05$)

Panel scale 1-8 (ex. 8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, 1 = extremely dry)

Table 4. Hunter L*, a*, and b* mean values for pork loin chops at 7, 14, 28, and 56 days of storage from pigs fed 0 or 1.0% CLA[a]

	Hunter Values											
	L*				a*				b*			
% CLA	Days postmortem				Days postmortem				Days postmortem			
	7	14	28	56	7	14	28	56	7	14	28	56
0	57.00	56.04	59.28	60.13	2.67	2.77	2.79	4.47	14.92	15.96	16.33	17.08
1.0	59.96	60.34	59.89	59.76	3.73	3.45	2.40	2.99	15.27	16.79	15.84	16.60
SEM	L* ± 1.50				a* ± .94				b* ± .68			

[a] Values are arithmetic means for eight observations

Table 5. Mean values for sensory panel results of pork loin chops at 7, 14, 28, and 56 days of storage from pigs fed 0 or 1.0% CLA[a]

Category	Day of Storage	0% CLA	SEM	1.0% CLA	SEM
Initial Juiciness	7	3.25[b]	.26	3.62[b]	.27
	14	4.12[c]	.27	4.46[c]	.26
	28	4.55[c]	.26	4.75[c]	.27
	56	4.20[c]	.27	3.46[b]	.26
Sustained Juiciness	7	3.63[b]	.23	3.71[b]	.24
	14	4.51[c]	.23	4.55[c]	.23
	28	4.70[c]	.23	4.77[c]	.24
	56	4.37[c]	.23	3.62[b]	.23
Initial Tenderness	7	4.04[b]	.23	4.37[b]	.24
	14	4.97[c]	.24	4.69[c]	.23
	28	5.44[c]	.23	5.11[c]	.24
	56	5.22[c]	.24	5.59[c]	.23
Overall Tenderness	7	4.55[b]	.23	4.39[b]	.23
	14	5.30[c]	.23	4.70[bd]	.23
	28	5.65[c]	.23	5.23[cd]	.23
	56	5.55[c]	.23	5.77[c]	.23
Pork Flavor	7	4.59[c]	.21	4.67	.21
	14	5.38[b]	.21	4.58	.21
	28	4.63[c]	.21	4.95	.21
	56	5.10[c]	.21	4.78	.21

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d] Values with different superscripts within a category and column indicate linear treatment effects ($P < .05$)

Panel scale 1-8 (ex. 8 = extremely juicy, 7 = very juicy, 6 = moderately juicy, 5 = slightly juicy, 4 = slightly dry, 3 = moderately dry, 2 = very dry, 1 = extremely dry)

Figure Legends

Figure 1. Subjective color, firmness and marbling and percent lipid mean values of pork loin chops at one day postmortem from pigs fed CLA

Figure 2. Percentage cook loss mean values of pork loin chops at one day postmortem from pigs fed CLA

Figure 3. Percentage cook loss and percentage drip loss mean values of pork loin chops at 7, 14, 28, and 56 days of storage from pigs fed 0 or 1.0% CLA

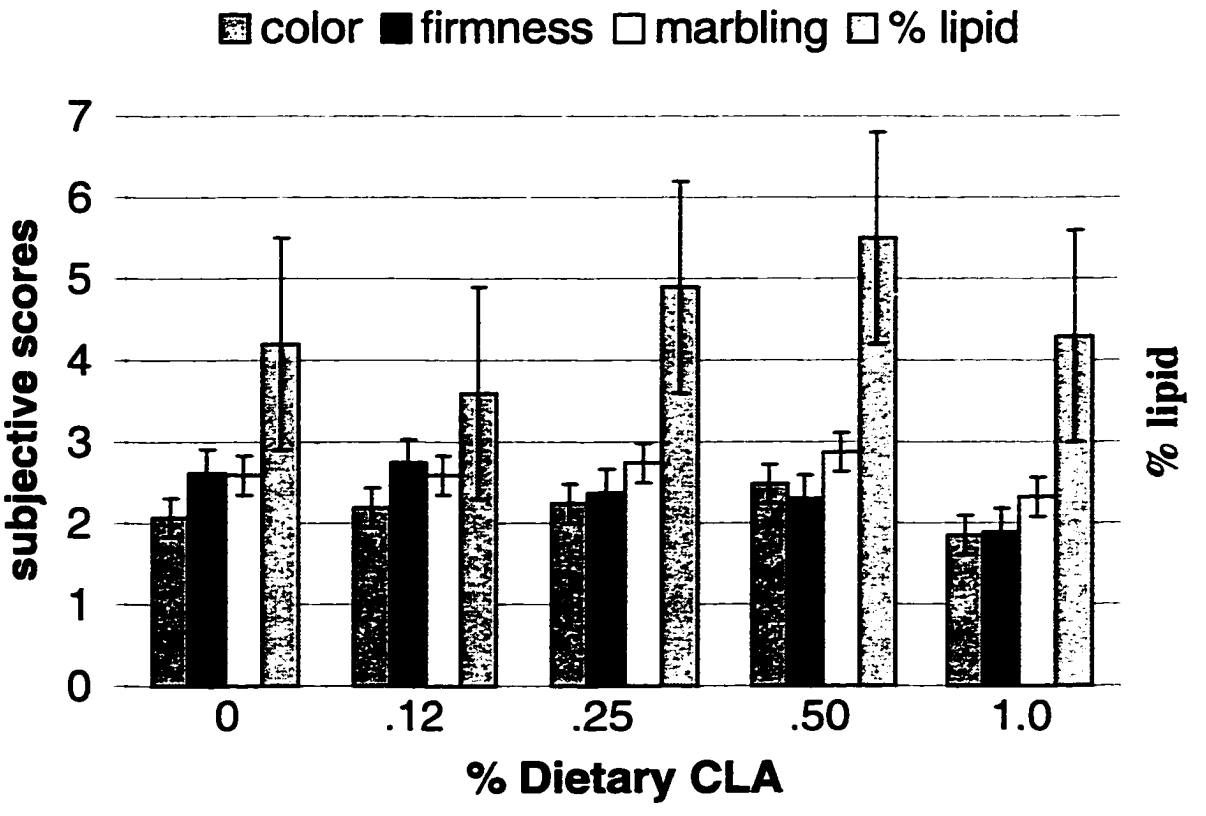
Figure 4. Sensory difference from control mean values for fresh pork patties at 1,3, and 7 days of storage from pigs fed CLA

Figure 5. Percentage cook loss mean values of fresh pork patties at 1, 3, and 7 days of storage from pigs fed CLA

Figure 6a. Hunter L* mean values for fresh pork patties at 1, 3, and 7 days of storage from pigs fed CLA

Figure 6b. Hunter a* mean values for fresh pork patties at 1, 3, and 7 days of storage from pigs fed CLA

Figure 6c. Hunter b* mean values for fresh pork patties at 1, 3, and 7 days of storage from pigs fed CLA

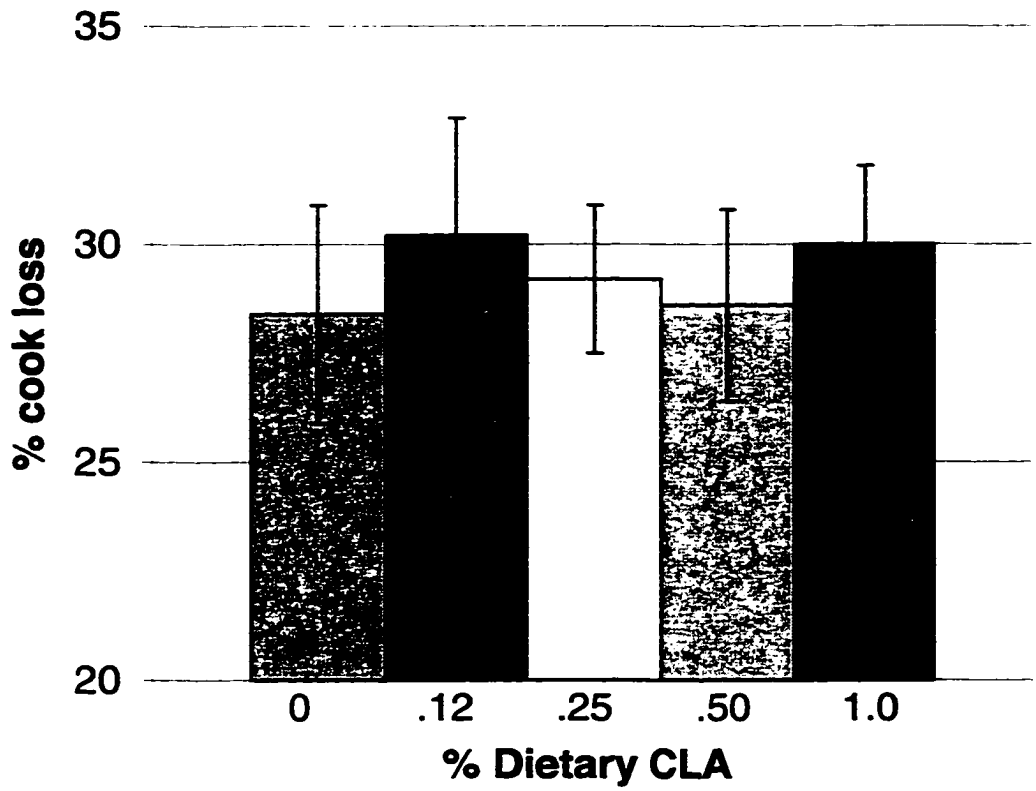


[a] NPPC standards used (scale 1-5)

1 = pale, pinkish gray; very soft; devoid to practically devoid

5 = dark purplish red; very firm; moderately abundant or greater

Figure 1.



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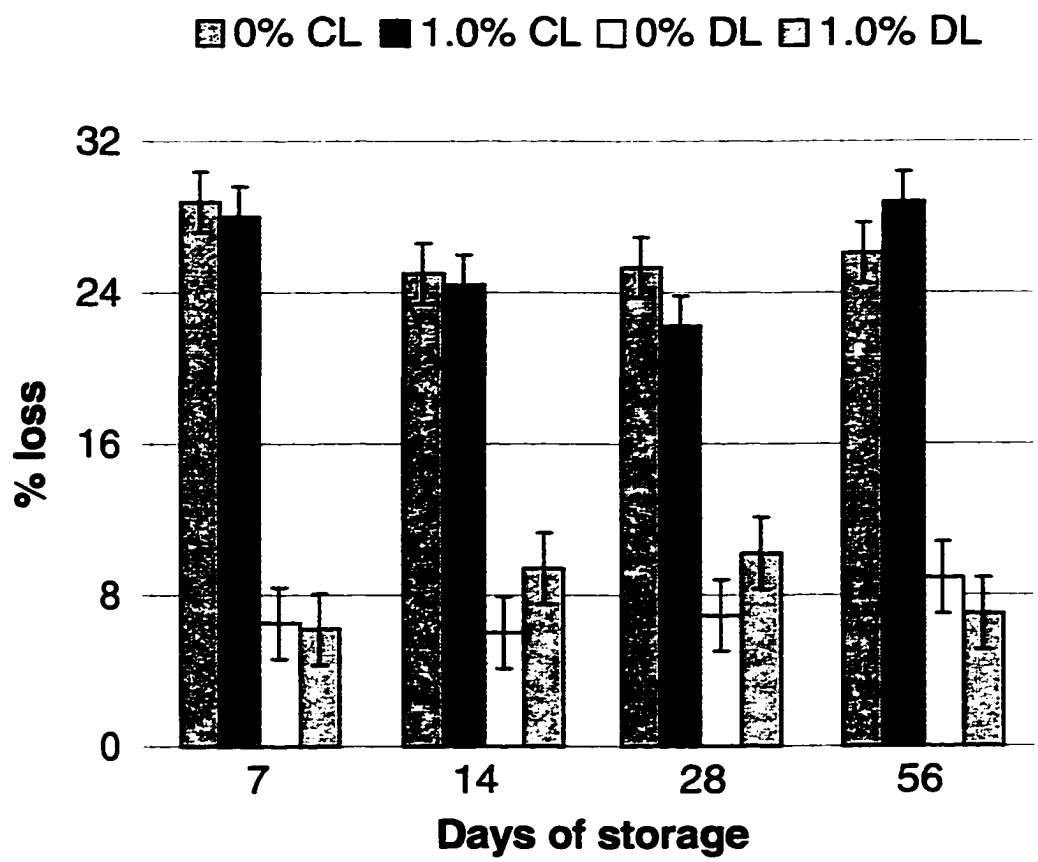


0% CL = 0% dietary CLA, cook loss
1.0% CL = 1.0% dietary CLA, cook loss
0% DL = 0% dietary CLA, drip loss
1.0% DL = 1.0% dietary CLA, drip loss

Figure 3.

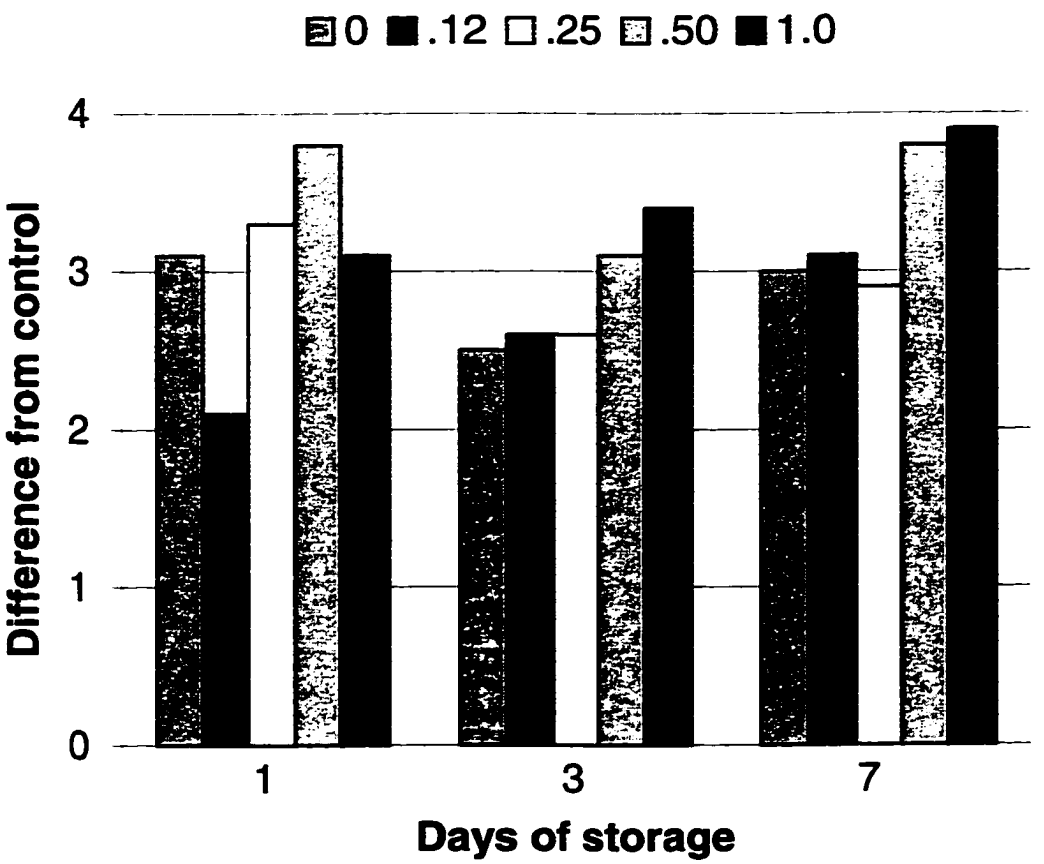
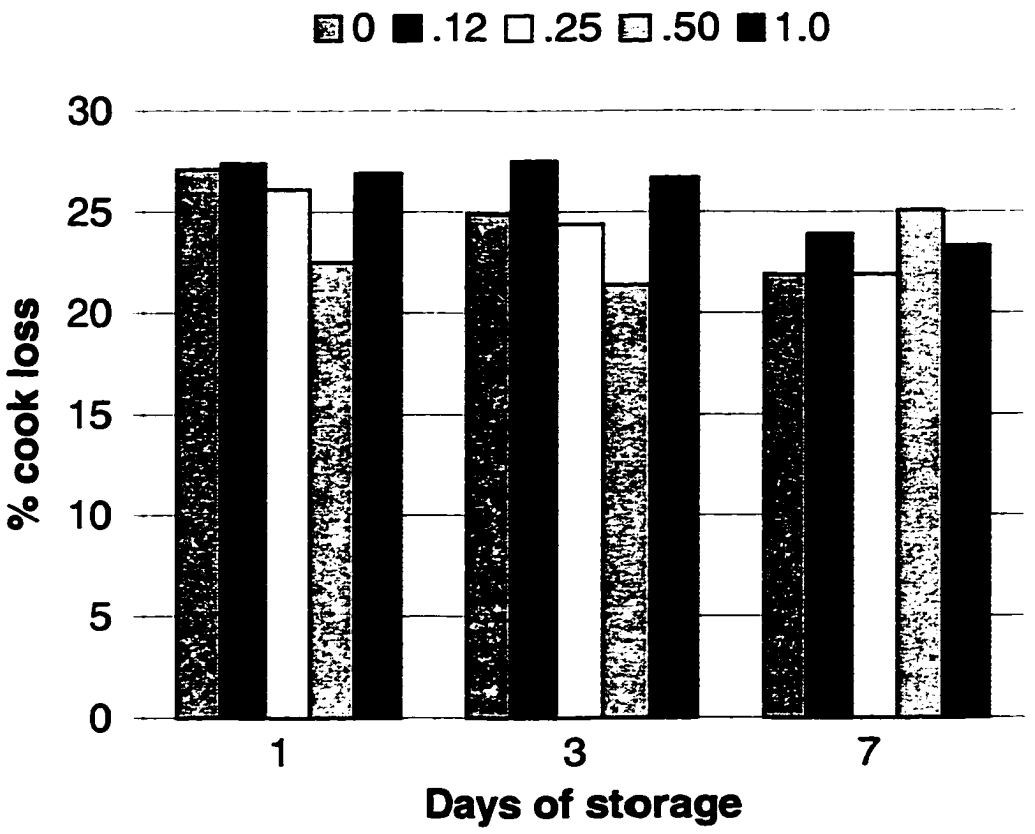

0 .12 .25 .50 1.0
Difference from control
4
3
2
1
0
1
3
7
Days of storage

Figure 4.



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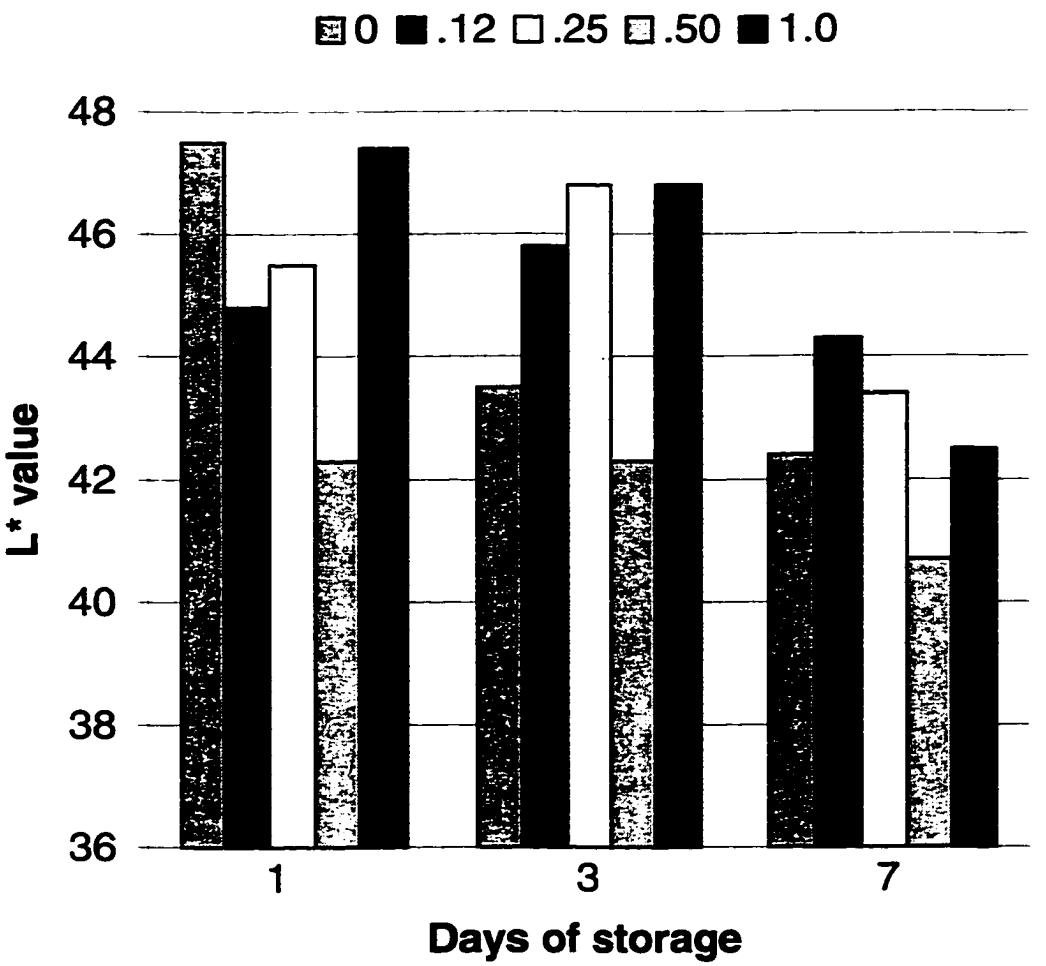

Figure 6a.

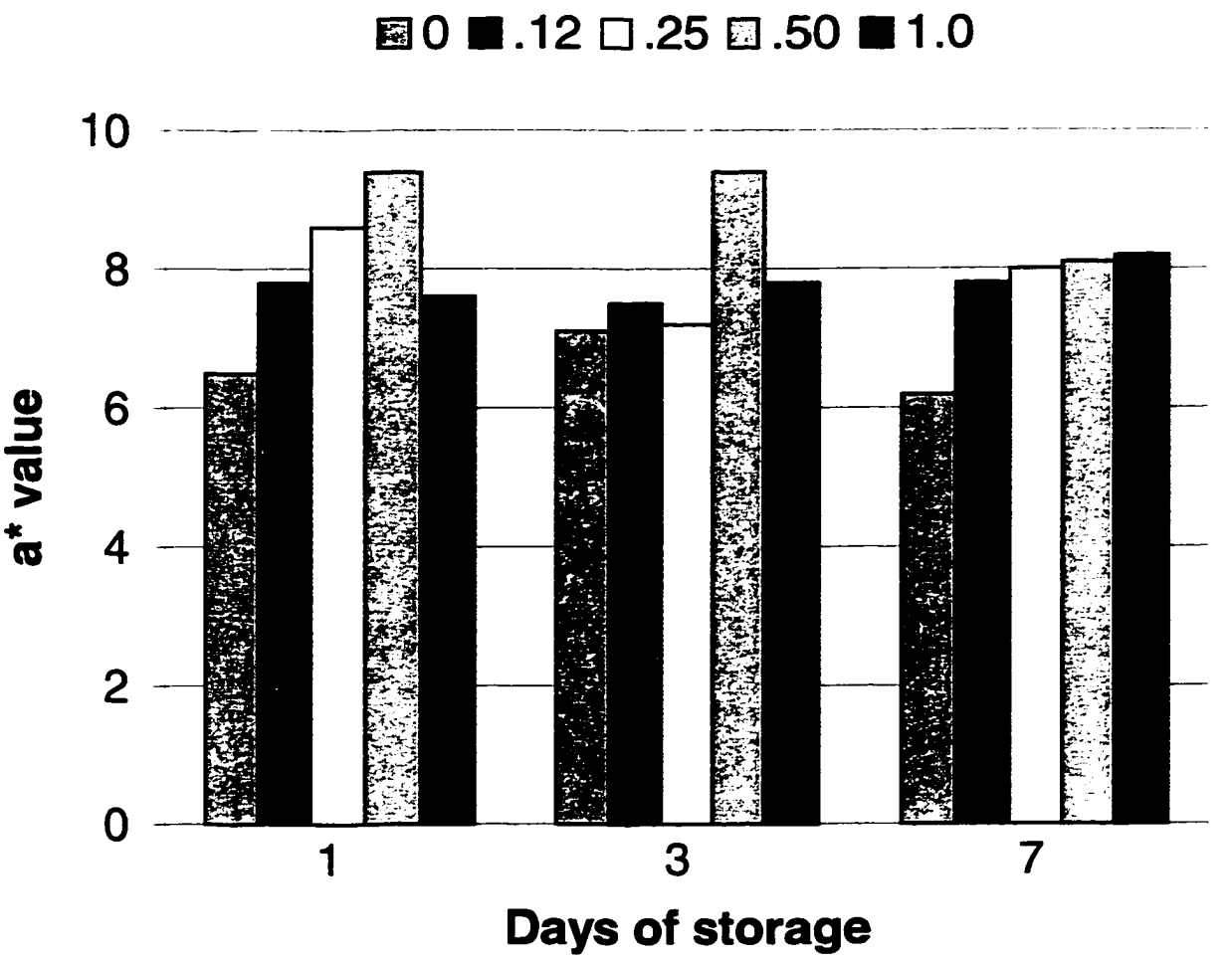

Figure 6b.

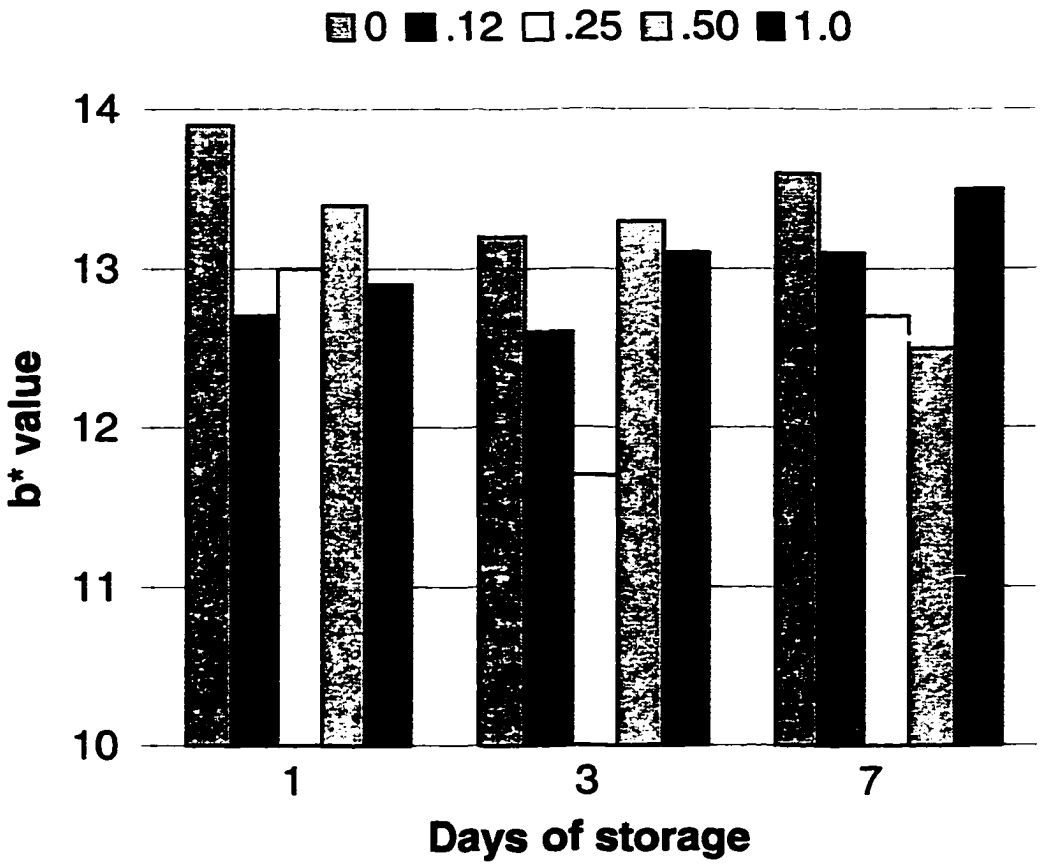

Figure 6c.

IMMUNE RESPONSE AND BLOOD CHEMISTRY OF BLOOD FROM CONJUGATED LINOLEIC ACID FED PIGS

A paper to be submitted to the Journal of Animal Science

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ABSTRACT

Immune function and blood chemistry parameters were measured in growing-finishing pigs (Yorkshire x Landrace x Duroc x Hampshire) fed varying amounts of conjugated linoleic acid (CLA). Blood was drawn from pigs at 52, 68, 91, and 114 kg live weight. Responses to stimulation with Con A mitogen tended to be reduced for all treatments as a factor of time. Nitric oxide (NO) concentration decreased over time, but was independent of treatment. Flow cytometry using fluorescent labeled monoclonal antibodies to the CD4, CD8, double positive CD4/CD8, and CD2 surface markers were used to determine lymphocyte sub-populations: CLA had no effect on lymphocyte subpopulation cell distribution. Most blood chemistry parameters were within the normal range for pigs. Calcium levels increased ($P< .001$) as the amount of CLA in the diet increased. Potassium ($P < .05$) and blood urea nitrogen ($P < .05$) levels decreased as the amount of CLA increased. Total protein demonstrated a quadratic effect of treatment ($P < .001$) with the .12, .25 and 50% CLA supplemented pigs having lower total protein than the controls or 1.0% CLA supplemented pigs. Blood lipid parameters indicated that total cholesterol ($P < .001$), triglycerides ($P < .001$), HDL ($P < .001$), and LDL ($P < .01$) all increased as the amount of CLA in the diet increased. Although there was a significant increase, none of the treatments exceeded the normal range of acceptability and triglycerides for all pigs were below the

normal range. CLA fed to growing-finishing pigs had little effect on their immune function or blood chemistry parameters. Thus, CLA can enhance pig performance without risk to swine health and disease.

Key words: Pigs, conjugated linoleic acid, immunology

INTRODUCTION

Conjugated dienoic derivatives of linoleic acid (CLA) are a mixture of geometric and positional isomers of linoleic acid (c-9, c-12-octadecadienoic acid). These isomers occur naturally in food (Ha et al., 1987, Ha et al., 1989, and Chin et al., 1992). CLA has been shown to inhibit mammary tumors in rats (Ip et al., 1994), prostate cancer in mice (Cesano et al., 1998) and to inhibit cancer cell proliferation in vitro (Schultz et al., 1992). CLA also has been shown to be a potent growth promoter in animals demonstrating both improved feed efficiency and increased weight gain (Chin et al., 1994, Dugan et al., 1997, Park et al., 1997, and Cook et al., 1999).

Several studies have been conducted to determine the effects of CLA on the immune system. Rats fed 0.5% CLA in the diet had enhanced macrophage phagocytosis and foot pad swelling in response to phytohemagglutinin, PHA (Cook et al., 1993). Mice fed either 0.3% or 0.9% CLA had increased lymphocyte proliferation in vitro in response to PHA but not Concanvalin A (Con A) or lipopolysaccharide (LPS). Production of IL-2 in these mice was also stimulated by CLA (Wong et al., 1997). Another study conducted with rats fed polyunsaturated fatty acid (PUFA) in combination with CLA found that the type of PUFA did not affect CLA reduction of basal tumor necrosis factor (TNF) production, but did

influence the effect of CLA on basal and LPS-induced IL-6 production (Turek et al., 1998). Because a stimulated immune system can adversely affect the performance of growing pigs, this study was undertaken to determine if immune stimulation occurs in pigs fed CLA during growing and finishing.

MATERIALS AND METHODS

Animals and Diets

Eight replications of five littermate barrows (Yorkshire x Landrace x Hampshire x Duroc) with an average initial weight of 26.3 kg were allotted at random to individual pens. Within replication, treatments of diets containing 0, .12, .25, .50, or 1.0% CLA were assigned at random. The source of CLA (PharmaNutrients, Oak Brook, IL) contained 60.5% CLA and was added to the diets at concentrations of .20, .42, .83, or 1.67% to provide the desired concentrations of CLA. The CLA was substituted for corn. The diets were initially formulated to contain 18.7% crude protein and 1.0% lysine. Diets were reformulated at about 3 week intervals (six formulations) to reduce crude protein and lysine content to 12.3% and .55% respectively, in the final finishing stage. Room temperature was maintained at about 18-21°C. Pigs were allowed ad libitum access to feed and water. Pigs were weighed and feed disappearance was determined at 14-day intervals.

Immune function

Blastogenesis was assayed using leukocytes from blood samples that were collected at 52, 68, 91, and 114 kg live weight using the T-cell mitogen Con A. Heparinized blood was diluted 1:10 in RPMI 1640 medium and 50 μg/ml gentamicin that contained 5 U/ml heparin supplemented with 10mM HEPES. Diluted blood (100 μl) was added in triplicate to

the 96-well flat bottomed tissue culture grade plate (Costar, Cambridge, MA) that also contained 100 µl of the mitogen Con A (5,10, 20 mg/ml final concentration) diluted in RPMI-1640 supplemented with 10mM HEPES and 50 µg/ml gentamicin (supplemented RPMI). The background wells contained only supplemented RPMI. Blood cultures were incubated for 72 hours at 37 °C in humidified CO_2 (5% CO_2, 95% air) incubator and pulsed with [^{3}H] thymidine (1µci/well) for the last 5 hours. At the end of incubation, the cultures were harvested onto glass-fiber filters (Skatron Instruments, Inc., Sterling VA) and counted by using a scintillation counter (LKB 1217 Rackbeta, Gaithersburg, MD). Sample radioactivity was normalized to 10^5 WBC/well based on WBC counts performed on whole blood using a Celltrack (Nova).

Culture supernates (from mitogen stimulated peripheral blood leukocytes) were tested for TNF and nitric oxide. The mitogens Con A (10 mg/ml) and LPS (5 mg/ml) were incubated with an equal volume of diluted blood (1:10) in 24-well tissue culture plates (Costar, Cambridge, MA) for 72 hours at 37 °C in humidified CO_2 (5%) incubator. The background wells contained supplemented RPMI. After incubation, the plates were centrifuged at 1200 rpm for 5 minutes, and the supernants were transferred to microcentrifuge tubes and frozen at –80 °C until subsequent analysis.

The nitric oxide concentrations were determined as nitrite by a microplate assay. Supernants were harvested from cultures of mitogen-stimulated lymphocytes prior to pulsing with [^{3}H] thymidine. Nitrate was reduced to nitrite using nitrate reductase. Fifty microliters of reduced sample aliquots were mixed with 100 µl of Griess reagent (0.5% sulfanilamide/0.05% naphthylethylene diamine dihydrochloride/2.5% H_3PO_4) and incubated

at 20 °C for 10 minutes. The optical density was measured in an ELISA microtiter plate reader (B10 Kinetics Reader, BIO-TEK instruments) at 550 nm against PBS as a blank. A sodium nitrite ($NaNO_2$) standard curve (0-100 μM) was generated in parallel. Results are expressed as the mean of nitrite concentrations in the supernatants of triplicate wells.

The positive percentages of CD4, CD8, and CD2 lymphocyte subsets were studied by fluorescent cell surface staining with monoclonal antibodies and analysis on an XL flow cytometer (Coulter Corporation Inc.). Monoclonal mouse antibodies to lymphocyte populations were purchased from VMRD, Pullman, WA, while fluorescene conjugated second step antibodies were purchased from Southern Biotechnology, Birmingham, AL. The anti-CD4 antibody (PT90A) was used with a second step of goat anti-mouse IgG2a-R-PE, and the anti-CD8 antibody (PT81B) was used with a second step of goat anti-mouse IgG2b-FITC to be used for double staining. Cells were also examined for CD2 staining using the antibody MSA4 and second step of goat anti-mouse IgG2a-R-PE in a separate tube. Mouse IgG2a and IgG2b,k-isotype standard antibodies (Southern Biotechnology) were used as control antibodies. Whole blood aliquots (100 μl) were suspended in 100 μl of cold PBS/Azide (0.1%) and incubated in the dark at 4 °C for 20 minutes with 5-10 μl of the monoclonal antibodies or isotypic control antibodies. The erythrocytes were lysed with freshly diluted lysing solution (ammonium chloride 8.02 g, sodium bicarbonate .84g, and EDTA disodium .37 g per 1 liter distilled water), for 10 minutes at room temperature. The remaining lymphocytes washed twice with PBS/Azide. The cells were fixed with 2% paraformaldehyde. The cells were analyzed on an XL flow cytometer.

Blood Chemistry

Blood chemistry tests were measured by using either a Hitachi 747 or Hitachi 917 automated clinical chemical analyzer at the University of Iowa medical school according to manufacturers instructions for testing each individual parameter (Boehringer Mannheim, Indianapolis, IN). The following abbreviated procedures to analyze sodium, potassium, chloride, blood urea nitrogen, creatinine, total proteins, albumin, calcium, inorganic phosphorus, glucose, uric acid, alkaline phosphotase, γ-glutamyl transferase, total bilirubin, direct bilirubin, creatine kinase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, cholesterol, triglycerides, and HDL-cholesterol are: 1) Sodium, potassium, and chloride which all carry a single charge were measured by electrical potential (electromotive force, EMF). The sodium and potassium electrodes were based on neutral carriers and the chloride electrode was based on an ion exchanger. 2) To measure blood urea nitrogen (BUN), urea was hydrolyzed by urease to form CO_2 and ammonia. Ammonia then reacted with α - ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD^+, the decrease in absorbance due to consumption of NADH was measured kinetically. 3) Creatinine was measured by a substrate triggered, rate-blanked method. In alkaline medium, creatinine forms a yellow-orange colored complex with picric acid. The rate of color formation was proportional to the concentration of creatinine present and was measured photometrically. 4) In proteins, a chelate was formed between one cupric ion and four to six nearby peptide bonds. The intensity of the color was proportional to the total number of peptide bonds undergoing reaction, thus indicating total protein. 5) Albumin binds selectively to bromcresol green (BCG) at a pH of approximately 4. 6) Calcium reacted with o-cresolphthalein complexone in the presence of 8-quinolinol salt to form a purple

chromophore. Total absorbance was measured at 570 nm. EDTA was then added to reverse the complexation of calcium with o-cresolphthalein complexone. A second absorbance reading was taken at 570 nm. Net absorbance was proportional to the amount of calcium in the specimen. 7) Inorganic phosphorus reacted with ammonium molybdate in an acidic solution to form ammonium phosphomolybdate. The ammonium phosphomolybdate was quantified in the ultraviolet range (340 nm), utilizing a sample blanked endpoint method. 8) Phosphorylation of glucose by ATP was catalyzed by hexokinase to form G-6-P and ADP. G-6-P was oxidized to 6-phosphogluconate in the presence of NADP by the enzyme glucose-6-phosphate dehydrogenase. The amount of NADPH formed during the reaction was equivalent to the amount of D-glucose in the specimen and measured photometrically by the increase in absorbance at 340 nm. 9) Uric acid was oxidized by the specific enzyme uricase to form allantoin and H_2O_2. The intensity of the red color formed was proportional to the uric acid concentration. p-Nitrophenylphosphate was hydrolyzed in the presence of magnesium ions by phosphatates to phosphate and p-nitrophenol. The rate of p-nitrophenol liberation was proportional to the alkaline phosphatase activity and was measured photometrically at 405 nm. 10) The rate of 5-amino-2-nitrobenzoate liberation (L-γ-glutamyl-3-corboxy-4-nitroanilide + glycylglycine → 5-amino-2-nitrobenzoate + L-γ-glutamyl-glycylglycine) was proportional to GGT activity and was measured photometrically at 405 nm. 11) Total bilirubin was coupled with a diazonium salt (DPD) in a strongly acid medium (pH 1-2). The intensity of the color of the azobilirubin produced was proportional to the total bilirubin concentration and was measured photometrically at 570 nm. Acidified sodium nitrite produces nitrous acid, which then reacts with sulfanilic acid to form a diazonium salt. The diazotized sulfanilic acid then reacts with bilirubin to form isomers of

azobilirubin. In the direct bilirubin assay, only conjugated bilirubin was converted by the diazotized sulfanilic acid. The intensity of the red color of azobilirubin was measured photometrically at 570 nm and was proportional to the direct (conjugated) bilirubin concentration. 12) The method of assaying creatine kinase activity included the use of creatine phosphate and ADP as substrates. The formation of NADPH in this reaction proceeds at the same rate as the formation of creatine in equimolar amounts. The rate of NADPH formation was proportional to CK activity and was measured photometrically at 340 nm. 13) α-Ketoglutarate reacts with L-aspartate in the presence of AST to form L-glutamate plus oxaloacetate. The indicator reaction utilized the oxaloacetate for a kinetic determination of NADH consumption. 14) α-Ketoglutarate reacted with L-alanine in the presence of ALT to form L-glutamate plus pyruvate. The indicator reaction utilized the pyruvate for a kinetic determination of NADH consumption. 15) Lactate dehydrogenase was measured as (L-lactate + $NAD^+ \leftrightarrows$ pyruvate + NADH + H^+) NAD and lactate are converted in equimolar amounts at the same rate. The rate at which NADH was formed was determined by an increase in absorbance at 340 nm and is directly proportional to enzyme activity. 16) All cholesterol esters present in serum were hydrolyzed quantitatively into free cholesterol and fatty acids by microbial cholesterol esterase. In the presence of oxygen, free cholesterol was oxidized by cholesterol oxidase to cholest-4-en-3-one and H_2O_2. The H_2O_2 reacted in the presence of peroxidase with phenol and 4-aminophenazone to form an o-quinone imine dye. The intensity of the color formed is proportional to the cholesterol concentration and was measured photometrically at 505 nm. 17) For the quantitative determination of tryglycerides, free glycerol was eliminated prior to hydrolysis of triglycerides in a preliminary reaction in which lipase and 4-aminophenazone were omitted. This reaction was followed by enzymatic

hydrolysis of triglycerides and determination of the liberated glcerol by a fully enzymatic colorimetric assay reaction. 18) The cholesterol concentration of HDL-cholesterol was determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with polythylen glycol (PEG) to the amino groups. Cholesterol esters were broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase. In the presence of oxygen, cholesterol was then oxidized by cholesterol oxidase to Δ^4 cholestenone and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide generated reacted with 4-aminophenazone and HDAOS (N-[2-hydroxy-3-sulfopropyl]-3,5-dimthoxanaline) to form a purple blue dye. The color intensity of this dye was directly proportional to the cholesterol concentration and was measured photometrically at 600 nm.

Statistical Analysis

Results were analyzed with the GLM procedures of SAS (1998) with the pig as the experimental unit. General linear models included main effects for treatment and replication. The model described linear and quadratic effects and treatment x weight interaction relationships.

RESULTS AND DISCUSSION

The number of white blood cells (Table 1) demonstrates a treatment by weight interaction ($P < .001$). White blood cells (WBC) were measured because they are the primary effector cells against infection and tissue damage. WBC numbers that are greatly elevated would indicate an acute infection or immune system dysfunction, whereas a decrease in number would indicate either immunodeficiency or an overwhelming infection that has depleted WBC stores. A general increase was observed in the number of white blood cells as pig weight increased to 91 kg. The controls demonstrated a significant

increase between 52 kg and 91 kg weights. No significant differences were observed in the .12% CLA treatments. The .25% CLA treated pigs had significantly increased white blood cell counts at 91 kg compared to 52 and 68 kg. The .50% CLA treated pigs were different between the 52 kg measurement and all other weights (68, 91 and 114 kg). The 1.0% CLA treated pigs were different at 68 kg from 91 and 114 kg only. The general trend for white blood cell numbers to increase only to 91 kg suggests that under these test conditions pigs were not exposed to new bacterial or viral agents in their environment. An increase in WBC number is a result of inducible hematopoiesis. Inducible hematopoiesis was greatest in the control, .25, .50 and 1.0% groups.

The number of monocytes per ml of blood was calculated by multiplying the percentage of monocytes x the number of white blood cells. Analysis of the number of monocytes indicated a highly significant ($P < .0001$) treatment x weight interaction with the 0, .12, .25 and 1.0% CLA treatments different at 52 kg compared to 68, 91 and 114 kg of live weight. The .50% CLA treatment was only different between 52 kg (higher) and 68 and 114 kg but not at 91 kg. The number of monocytes was higher at 52 kg than at 68, 91 and 114 kg.

The number of lymphocytes also demonstrated a highly significant ($P < .0001$) treatment x weight interaction with 52 kg having lower numbers than 91 and 114 kg for controls, .25 and 1.0% CLA treatments. Lymphocyte number for 52 kg was also lower than 68 and 114 kg for .12% CLA treatment and lower than 68, 91 and 114 kg for .50% CLA treatment.

The number of granulocytes demonstrated a highly significant ($P < .01$) treatment interaction with 52 kg having lower numbers than 91 kg for controls and .50% CLA

treatment. No other significant differences between treatments were determined. Taken together the observed increase in WBC number per ml of blood is due at first to expansion of lymphocytes and subsequently to a progressive increase in granulocytes.

In newborn piglets, the total peripheral white blood count of 4.2 – 9 x 10^6 / ml is made up of about 60% neutrophils and 38% lymphocytes (Mandel, 1982). This ratio is inverted by d 10 after birth (60% lymphocytes, 38 % neutrophils). de Gruijter et al. (1990) determined that Ficoll-paque preparations of peripheral blood of 8 – 10 week old (≈ 20 kg) Yorkshire pigs was about 10 – 15% monocytes and 60 – 75% lymphocytes. Our study, which used FACS analysis without prior Ficoll-paque separation, indicated that the % monocytes decreased with the age of the pig from 19.1% at 52 kg to 8.5% at 114 kg. The decreased percentage of monocytes was mirrored by an increased percentage of lymphocytes with pigs at 52 kg expressing 49.4% and 58.8% lymphocytes at 114 kg. The granulocytes made up the remaining percentage of WBC. This fluctuation in leukocyte subpopulations reflects an animals normal response to environmental exposure to bacteria and viruses.

Basal production of nitric oxide (Table 2) indicated that production was lower at 114 kg in both control and CLA treated pigs ($P < .001$) than at 52, 68, or 91 kg. In general, this data reflects that over time nitrate is decreasing in blood plasma. We were unable to significantly stimulate nitric oxide production with Con A or LPS, likely because of the very high levels of plasma nitric oxide reported. Stimulation was not measurable due to high initial levels of nitrite found in plasma. Plasma nitrate levels come from oxidation of NO produced by both constitutive and inducible NOS within various cells.

The number of T-cell subpopulations of blood (Table 3) were analyzed as CD2 (T cells and NK cells), CD4 (helper subset), CD8 (cytotoxic subset), and CD4/8 (double

positive, memory cells). Data was collected as percentage of lymphocytes but analyzed as number per ml of blood since WBC number varied by pig weight. There were treatment by weight interactions for each subpopulation; CD4 ($P < .0001$), CD8 ($P < .0001$), CD4/8 ($P < .001$) and CD2 ($P < .0001$). CD4 number was lower at 52 kg than at 68, 91 and 114 kg for both controls and blood from CLA supplemented pigs.

CD8 number was not different over weight for control animals, however, the treated groups all exhibited differences in CD8 number. The .12% CLA group exhibited a lower CD8 value at 52 kg than at 68 and 114 kg. The .25 and .50% CLA group exhibited a lower CD8 number at 58 kg than at 68, 91 and 114 kg. In the 1.0% CLA group 52 kg demonstrated a lower CD8 number at 52 kg than at 91 kg only.

The number of CD4/8 double positive T-cells for controls indicated a difference between 52 kg and 91 kg only, with 52 kg having lower numbers of CD4/8 cells than 91 kg. The .12% treatment displayed lower numbers of CD4/8 cells at 52 kg than at 68 and 114 kg. The .25% treatment indicated the same difference as the controls with 52 kg having lower CD4/8 numbers than did 91 kg. In the .50% CLA treatment 68, 91 and 114 kg samples had higher CD4/8 numbers than did 52 kg. The 1.0% CLA treatment demonstrated a difference with 52 kg demonstrating lower CD4/8 numbers than did 91 and 114 kg. Thus, the double positive memory cells increased with time (weight gain) for all treatments as would be expected for healthy pigs responding to environmental flora and organisms.

The number of CD2 T-cells exhibited no differences between the controls at any live weight. The .12% CLA treatment indicated a difference in which 52 kg had significantly lower CD2 cell numbers than did 68 kg. Both .25 and .50% CLA treatments exhibited lower

CD2 cell numbers at 52 kg than at 68, 91 and 114 kg. The 1.0% CLA treatment indicated lower CD2 numbers at 52 kg than at 91 and 114 kg.

The T-cell subpopulations (CD4, CD8, CD4/8 and CD2) were also reported as a percentage of lymphocytes in Table 4. These percentages however, do not clearly indicate changes in actual numbers due to the increased number of WBC and lymphocytes.

Pigs have been used for many years to study various human diseases. One of the reasons that pigs are used is that many of their physiological systems are similar. The immune system is one physiological system of particular interest with the growing interest in animal welfare and xenotransplant use of swine for humans. A previous part of this study (Thiel-Cooper et al. 1999) investigated the effects of CLA on pig performance and demonstrated that average daily gain and gain / feed were significantly improved. This study found that feeding conjugated linoleic acid to growing-finishing pigs had few effects on the immune responses measured. The lack of response may be due to the fact these pigs were allowed to acquire normal immunity up to 26 kg. At 26 kg they were first introduced to CLA in their diets. This addition did not affect the number of white blood cells, monocytes or lymphocytes. The percentage of monocytes and lymphocytes reported in this study also are similar to those reported in other studies. The T-cell subpopulations (CD4, CD8, CD4/8 and CD2) were different at the lightest live weight. This difference is also supported by other studies that indicate that as pigs age these T-cell populations either increase or decrease. This study demonstrated that CLA fed to growing-finishing pigs does not compromise their immune system.

Blood chemistry (SMAC) results were recorded at 52, 68, 91 and 114 kg live wt. Measurements were made for sodium (Na), potassium (K), chloride (Cl), carbon dioxide

(CO_2), balance (Bal), blood urea nitrogen (BUN), creatine (Creat), total protein (T. Prot), albumin (Alb), calcium (Ca), inorganic phosphate (In Phos), glucose, uric acid (uric), alkaline phosphotase (Alk Phos), γ-glutamyl transpeptidase (GGT), total bilirubin (T Bil), direct bilirubin (D Bil), creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LD), cholesterol, triglycerides, high density lipoproteins (HDL), and low density lipoproteins (LDL).

Results for Na, Cl, CO_2, Bal, Creat, Alb, Glu, Alk Phos, GGT, T Bil, D Bil, CK, ALT, In Phos, AST and LD presented in Table 7, Table 7a, Table 7b, and Table 7c were within the normal ranges for pigs and demonstrated no treatment differences.

Four categories, K, BUN, T Prot, and Ca demonstrated significant linear treatment effects. As the amount of CLA in the diet increased the concentration of K decreased ($P < .05$). A severely decreased concentration of potassium occurs as a result of either a low intake over a period of time or an increased loss of potassium through diarrhea. An increase in secretion of adrenal steroids, primarily aldosterone, also results in excessive potassium loss. Although K demonstrated a linear treatment decrease the levels were not outside the normal reference range for pigs.

The concentration of blood urea nitrogen (BUN) also decreased ($P < .05$) as the amount of dietary CLA increased. Urea is the characteristic nitrogenous end product of protein catabolism in mammals. Ingested proteins are hydrolized to amino acids in the intestines. The absorbed amino acids are carried to the liver and other tissues where they may be utilized for the synthesis of new proteins, converted to other compounds, or utilize for energy. When catabolized, the amino group ends up in urea and is excreted by the kidneys. Decreased blood urea nitrogen therefore suggests that less protein catabolism

occurred with increased amounts of dietary CLA supplementation. Results of another portion of this study, indicated that there was a trend to increased loin muscle ($P = .07$). This increase in loin muscle may be due to reduced muscle catabolism as measured by decreased blood urea nitrogen.

The concentration of total protein demonstrated a linear treatment effect ($P < .003$). Determination of total protein concentration supplies limited information except in conditions relating to changes in plasma or fluid volume. Albumin is more commonly used to determine health status and no treatment effects were noted for albumin concentration in this study.

Calcium concentration increased ($P < .002$) with increased amounts of CLA supplementation. Although not outside the normal reference ranges for pigs this increase in Ca may cause stimulation in secreation of calcitonin. Calcitonin in turn acts upon bone osteoclasts and inhibits their dissolution of bone. The earlier findings of this study in which, increased amounts of bone were measured with increased dietary CLA supplementation may be explained by this inhibition of bone resorption.

Blood lipid results (Table 5) indicated significant differences between treatments for cholesterol ($P < .001$), triglycerides ($P < .001$), HDL ($P < .001$), and LDL ($P < .01$). Cholesterol was higher for 1.0% CLA supplemented pigs than for controls, .12 and .50% CLA supplemented pigs. The .12% CLA supplemented pigs exhibited lower cholesterol than did the .25 or 1.0% supplemented pigs. These differences in cholesterol values were not outside the normal reference ranges for pigs. Triglyceride levels increased significantly from .12% to 1.0% CLA supplementation, however controls were not different from any of the CLA treatments. HDL concentrations were higher for controls, .25 and 1.0% CLA than for

.12 and .50% CLA supplemented pigs. LDL concentrations for controls were lower than those for .25, .50 and 1.0% CLA supplemented pigs, also .12% CLA supplemented LDL levels were lower than .25 and 1.0% CLA supplemented pigs. Both HDL and LDL levels although different between treatments were not outside the normal reference ranges for pigs. Triglyceride levels however, were well below the normal references ranges for pigs for all treatments. The most likely reason for these low levels of triglycerides is due to their genetics.

This study demonstrated that feeding CLA to growing-finishing pigs (26 – 114 kg) had few effects on the blood chemistry components measured. The differences in blood urea nitrogen and calcium may positively effect the retention of protein and bone. Although treatment differences were found the values for K, BUN, Ca and T Prot were well within the expected / accepted ranges for disease free pigs.

IMPLICATIONS

This study demonstrated that growing-finishing pigs fed conjugated linoleic acid grew faster and more efficiently without having a compromised immune system or producing any pathological or disease state. There seems to be a large variation in immune response and blood chemistry by swine breed. Determination of the breed or breed cross that duplicates human atherosclerosis and other disease states most closely could provide excellent study models. Further study to determine the effects of long term feeding of CLA to pigs may provide excellent information relating to atherosclerosis in humans.

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Table 1. Number (x 10^6 / ml blood) of white blood cells, monocytes, lymphocytes and granulocytes for growing-finishing pigs fed differing amounts of CLA[a]

Component	Live wt (kg)	% CLA Added 0	.12	.25	.50	1.0
WBC						
	52	14.83 ± 1.14^{b}	13.79 ± 1.14	15.09 ± 1.14^{b}	14.14 ± 1.14^{b}	14.55 ± 1.14^{bc}
	68	15.58^{bc}	14.86	14.20^{b}	17.78^{c}	13.14^{b}
	91	18.18^{c}	14.71	18.91^{c}	20.13^{c}	17.23^{c}
	114	15.94^{bc}	16.71	17.29^{bc}	18.40^{c}	16.26^{c}
Monocytes						
	52	$2.96 \pm .19^{b}$	$3.02 \pm .19^{b}$	$2.97 \pm .19^{b}$	$2.23 \pm .19^{b}$	$2.68 \pm .19^{b}$
	68	1.78^{cd}	1.44^{c}	1.25^{c}	1.70^{c}	1.67^{cd}
	91	2.06^{c}	1.59^{c}	2.14^{d}	1.96^{bc}	1.98^{c}
	114	1.44^{cd}	1.41^{c}	1.52^{c}	1.48^{c}	1.37^{d}
Lymphocytes						
	52	$7.79 \pm .69^{b}$	$6.80 \pm .69^{b}$	$7.12 \pm .69^{b}$	$6.94 \pm .69^{b}$	$7.08 \pm .69^{b}$
	68	9.30^{bc}	8.97^{c}	8.27^{bd}	9.97^{c}	7.33^{b}
	91	10.10^{c}	8.29^{bc}	10.69^{c}	10.91^{c}	10.12^{c}
	114	9.73^{c}	9.69^{c}	9.74^{cd}	10.66^{c}	9.97^{c}
Granulocytes						
	52	$4.21 \pm .61^{b}$	$4.38 \pm .61$	$5.30 \pm .61$	$5.16 \pm .62^{b}$	$5.00 \pm .61$
	68	4.60^{bc}	4.48	4.71	5.80^{bc}	4.57
	91	6.21^{c}	4.65	6.32	7.40^{c}	5.35
	114	4.72^{bc}	5.58	6.01	6.23^{bc}	4.92

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d] Values with different superscripts within a category and column indicates treatment effects ($P < .01$)

Table 2. Basal concentration of nitric oxide (nmol nitrite / ml of supernate) for growing-finishing pigs fed differing amounts of CLA[a]

Live wt (kg)	% CLA Added				
	0	.12	.25	.50	1.0
52	707.9 ± 14.1[b]	741.8 ± 14.1[b]	684.9 ± 14.1[b]	719.7 ± 14.1[b]	718.2 ± 14.1[b]
68	791.2 ± 14.1[c]	788.3 ± 14.1[c]	775.5 ± 15.1[c]	786.9 ± 14.1[c]	790.8 ± 15.1[c]
91	734.3 ± 14.1[b]	743.3 ± 14.1[b]	753.4 ± 16.4[c]	740.8 ± 15.1[d]	739.4 ± 14.1[c]
114	391.0 ± 14.1[d]	412.6 ± 14.1[d]	403.1 ± 14.1[d]	381.3 ± 14.1[e]	389.5 ± 14.1[d]

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d,e] Values with different superscripts within a column indicate treatment effects ($P < .01$)

Table 3. T-cell subpopulations in blood, expressed as the actual number ($x10^6$ / ml blood) of positive lymphocytes staining with antibody for CD4, CD8, CD4/8 (double positive), and CD2 for growing-finishing pigs fed differing amounts of CLA[a]

	Live wt (kg)	% CLA Added				
		0	.12	.25	.50	1.0
CD4						
	52	.67 ± .12[b]	.45 ± .12[b]	.55 ± .12[b]	.45 ± .12[b]	.61 ± .12[b]
	68	1.33[c]	1.23[c]	1.13[c]	1.24[c]	.97[c]
	91	1.07[c]	.90[d]	1.01[c]	1.07[c]	.98[c]
	114	1.02[c]	1.02[cd]	1.04[c]	1.23[c]	1.00[c]
CD8						
	52	1.29 ± .26	.91 ± .26[b]	.86 ± .26[b]	.98 ± .26[b]	1.36 ± .26[b]
	68	1.87	1.77[c]	1.68[c]	2.19[c]	1.55[bc]
	91	1.92	1.52[bc]	1.99[c]	2.01[c]	2.22[c]
	114	1.82	1.84[c]	1.57[c]	2.25[c]	1.87[bc]
CD4/8						
	52	.48 ± .10[b]	.37 ± .10[b]	.38 ± .10[b]	.32 ± .10[b]	.46 ± .10[b]
	68	.64[bc]	.71[c]	.60[bc]	.68[c]	.56[bc]
	91	.84[c]	.64[c]	.74[c]	.64[c]	.80[c]
	114	.62[bc]	.72[c]	.62[bc]	.68[c]	.82[c]
CD2						
	52	3.37 ± .49	3.03 ± .49[b]	2.59 ± .49[b]	2.64 ± .49[b]	3.04 ± .49[b]
	68	4.63	4.97[c]	4.40[c]	5.37[c]	3.66[bc]
	91	4.69	3.88[bc]	5.11[c]	4.85[c]	4.90[c]
	114	4.25	3.75[bc]	4.36[c]	4.95[c]	4.38[c]

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d] Values with different superscripts within a category and column indicate treatment effects ($P < .01$)

Table 4. Percent T-cell subpopulations in blood, expressed as the percentage of positive lymphocytes staining with antibody for CD4, CD8, CD4/8 (double positive), and CD2 for growing-finishing pigs fed differing amounts of CLA[a]

		% CLA Added				
	Live wt (kg)	0	.12	.25	.50	1.0
CD4						
	52	8.44 ± .94[c]	7.01 ± .93[b]	7.53 ± .93[b]	6.40 ± .93[b]	8.23 ± .93[c]
	68	14.36[b]	13.98[c]	13.79[c]	12.39[c]	13.18[b]
	91	10.45[c]	10.84[d]	9.31[bd]	9.71[c]	9.55[c]
	114	10.63[c]	10.98[d]	10.76[d]	11.63[cd]	10.26[c]
CD8						
	52	15.36 ± 2.02	12.21 ± 1.98[b]	11.86 ± 1.98[b]	12.55 ± 1.98[b]	18.00 ± 1.98
	68	20.07	19.94[c]	19.78[cd]	21.59[c]	22.10
	91	18.84	17.98[c]	18.96[cd]	18.09[c]	20.81
	114	19.25	19.01[c]	16.02[bd]	20.83[c]	17.96
CD4/8						
	52	6.12 ± 1.01	5.23 ± .99	5.28 ± .99	4.41 ± .99	6.70 ± .99
	68	7.01	8.03	7.43	6.95	7.66
	91	8.46	8.00	7.25	6.08	7.74
	114	6.89	7.45	6.66	6.31	7.70
CD2						
	52	41.31 ± 3.62[b]	42.06 ± 3.62[c]	35.75 ± 3.62[b]	36.51 ± 3.62[c]	41.64 ± 3.62[b]
	68	51.89[c]	55.81[b]	52.23[c]	54.08[b]	51.84[c]
	91	46.41[bc]	45.75[c]	48.35[c]	44.13[c]	47.36[bc]
	114	44.89[bc]	38.64[c]	45.09[bc]	46.31[bc]	43.40[bc]

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d] Values with different superscripts within a category and column indicate treatment effects ($P < .01$)

Table 5. Blood lipid mean values (mg / dl) and standard error from growing-finishing pigs fed differing amounts of CLA[a]

	% Dietary CLA				
Component	0	.12	.25	.50	1.0
Cholesterol	84.6 ± 1.4^{bd}	82.3 ± 1.4^{b}	88.1 ± 1.4^{cd}	85.0 ± 1.5^{b}	90.3 ± 1.4^{c}
Triglyceride	17.8 ± 1.8^{bc}	13.6 ± 1.8^{b}	14.2 ± 1.8^{b}	16.2 ± 1.8^{b}	22.3 ± 1.8^{c}
HDL	36.1 ± 0.7^{b}	33.7 ± 0.7^{c}	35.9 ± 0.7^{b}	34.0 ± 0.7^{c}	37.2 ± 0.7^{b}
LDL	45.0 ± 1.0^{b}	45.9 ± 1.0^{bd}	49.3 ± 1.0^{c}	47.7 ± 1.0^{cd}	48.6 ± 1.0^{c}

[a] Values are arithmetic means ± SEM for eight observations

[b,c,d] Values with different superscripts within a row indicate linear treatment effects ($P < .01$)

Table 6. Potassium, calcium, blood urea nitrogen and total protein mean values and standard error from growing-finishing pigs fed varying amounts of CLA[a]

Component	Pig wt (kg)	% Dietary CLA					Reference Range[b]
		0	.12	.25	.50	1.0	
Potassium							
(meq / L)	52	7.2 ± .3	6.9 ± .3	6.9 ± .3	7.2 ± .3	7.0 ± .3	4.0 – 7.0
	68	6.4	6.7	6.3	6.3	6.0	(meq / L)
	91	6.6	6.7	5.9	6.0	6.0	
	114	5.7[c]	5.3[cd]	5.4[cd]	5.2[cd]	4.7[d]	
Calcium							
(mg / dl)	52	10.6 ± .1[d]	10.5 ± .1[d]	10.2 ± .1[c]	10.7 ± .1[d]	10.6 ± .1[d]	8.6 – 11.7
	68	10.5	10.5	10.4	10.6	10.6	(mg / dl)
	91	10.4[d]	10.5[cd]	10.4[d]	10.5[cd]	10.7[c]	
	114	10.6[d]	10.8[cd]	10.6[d]	10.7[cd]	10.9[c]	
Blood Urea Nitrogen							
(mg / dl)	52	11 ± 1	11 ± 1	11 ± 1	10 ± 1	10 ± 1	10 - 34
	68	12[d]	14[c]	12[d]	12[d]	12[d]	(mg / dl)
	91	14[c]	12[cd]	11[d]	12[cd]	11[d]	
	114	15[c]	12[d]	13[cd]	12[d]	12[d]	
Total Protein							
(g / dl)	52	5.4 ± .1[c]	5.2 ± .1[cd]	5.1 ± .1[d]	5.1 ± .1[cd]	5.2 ± .1[cd]	5.2 – 8.3
	68	5.8	5.7	5.7	5.5	5.7	(g / dl)
	91	6.4[d]	6.2[cd]	6.3[d]	6.0[c]	6.4[d]	
	114	6.5	6.5	6.4	6.4	6.6	

[a] Values are arithmetic means ± SEM for eight observations

[b] Reference ranges adapted from Leman, (1992)

[c,d] Values with different superscripts within a row indicate linear treatment effects ($P < .05$)

Table 7a. Sodium, chloride, carbon dioxide, balance, creatinine, and albumin mean values and standard error from growing-finishing pigs fed varying amounts of CLA[a]

		% Dietary CLA					
Component	Pig wt (kg)	0	.12	.25	.50	1.0	Reference Range[b]
Sodium (meq / L)	52	141 ± 1	142 ± 1	140 ± 1	142 ± 1	142 ± 1	135 -150
	68	158	155	157	158	160	(meq / L)
	91	141	143	142	144	143	
	114	144	145	145	145	145	
Chloride (meq / L)	52	102 ± 1	104 ± 1	103 ± 1	104 ± 1	104 ± 1	95 –110
	68	115	113	115	115	117	(meq / L)
	91	104	104	104	104	103	
	114	103	103	103	103	102	
Carbon Dioxide (meq / L)	52	26 ± 1	26 ± 1	27 ± 1	28 ± 1	26 ± 1	NA[c]
	68	26	26	28	26	26	
	91	29	28	29	29	28	
	114	27	28	28	28	27	
Balance (meq / L)	52	13 ± 1	12 ± 1	10 ± 1	11 ± 1	13 ± 1	NA[c]
	68	16	16	14	18	16	
	91	9	10	11	11	11	
	114	14	15	14	14	16	
Creatinine (mg / dl)	52	1.0 ± .1	1.0 ± .1	1.0 ± .1	.9 ± .1	1.0 ± .1	.5 – 2.7
	68	1.1	1.0	1.1	1.2	1.1	(mg / dl)
	91	1.4	1.3	1.5	1.5	1.6	
	114	1.6	1.7	1.7	1.7	1.7	
Albumin (g / dl)	52	3.9 ± .1	3.9 ± .1	3.7 ± .1	4.0 ± .1	3.9 ± .1	3.0 – 4.5
	68	4.2	4.4	4.4	4.1	4.4	(g / dl)
	91	4.5	4.6	4.5	4.3	4.6	
	114	4.5	4.6	4.5	4.4	4.6	

[a] Values are arithmetic means ± SEM for eight observations

[b] Reference ranges adapted from Leman, (1992)

[c] NA = reference ranges not available for these parameters

Table 7b. Inorganic phosphorus, glucose, alkaline phosphatase, γ-glutamyl transpeptidase, and total bilirubin mean values and standard error from growing-finishing pigs fed varying amounts of CLA[a]

Component	Pig wt (kg)	% Dietary CLA 0	.12	.25	.50	1.0	Reference Range[b]
Inorganic Phosphorus (mg / dl)	52	9.2 ± .2	9.2 ± .2	9.0 ± .2	9.2 ± .2	9.2 ± .2	5.8 – 13.8
	68	8.9	8.8	8.8	8.7	9.0	(mg / dl)
	91	8.2	8.5	8.2	8.1	8.3	
	114	7.4	7.8	7.5	7.1	7.5	
Glucose (mg / dl)	52	113 ± 3	112 ± 3	114 ± 3	113 ±3	110 ± 3	65 - 150
	68	103	110	104	116	113	(mg / dl)
	91	101	103	103	100	103	
	114	101	91	96	91	93	
Alkaline Phosphatase (U / L)	52	264 ± 49	255 ± 50	251 ± 50	247 ± 51	251 ± 49	142 - 891
	68	191	185	172	179	166	(U / L)
	91	334	160	155	155	145	
	114	145	143	134	126	274	
γ-glutamyl Transpeptidase (U / L)	52	66 ± 6	67 ± 6	67 ± 6	62 ± 6	61 ± 6	10 - 50
	68	38	33	31	31	30	(U / L)
	91	35	32	29	30	29	
	114	34	32	30	29	29	
Total Bilirubin (mg / dl)	52	.1 ± .04	.1 ± .04	.1 ± .04	.1 ± .04	.1 ± .04	0.0 –1.0
	68	.2	.2	.2	.2	.2	(mg / dl)
	91	.2	.3	.1	.1	.1	
	114	.1	.2	.1	.2	.1	

[a] Values are arithmetic means ± SEM for eight observations

[b] Reference ranges adapted from Leman, (1992)

Table 7c. Creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase mean values and standard error from growing-finishing pigs fed varying amounts of CLA[a]

Component	Pig wt (kg)	% Dietary CLA 0	.12	.25	.50	1.0	Reference Range[b]
Creatine Kinase (U / L)	52	9106 ± 1307	4835 ± 1331	5281 ± 1331	9550 ± 1360	5452 ± 1307	100 - 2500 (U / L)
	68	1980	2790	2980	1299	2130	
	91	2865	2743	3544	3160	2968	
	114	1686	1613	1109	1146	2427	
Lactate Dehydrogenase (U / L)	52	1233 ± 128	851 ± 131	797 ± 133	1229 ± 133	905 ± 128	250 - 600 (U / L)
	68	623	679	719	566	738	
	91	711	722	818	860	934	
	114	532	558	477	531	818	
Aspartate Aminotransferase (U / L)	52	44 ± 8	40 ± 8	29 ± 8	41 ± 8	35 ± 8	10 – 100 (U / L)
	68	49	40	47	27	35	
	91	46	40	48	34	39	
	114	33	25	24	26	36	
Alanine Aminotransferase (U / L)	52	32 ± 3	35 ± 3	34 ± 3	36 ± 3	34 ± 3	15 - 46 (U / L)
	68	37	38	36	36	43	
	91	37	36	33	35	37	
	114	37	34	32	33	37	

[a] Values are arithmetic means ± SEM for eight observations

[b] Reference ranges adapted from "Diseases of Swine" (19)

GENERAL CONCLUSIONS

From the studies contained in this body of work, several conclusions can be made. First, it can be concluded that conjugated linoleic acid has positive effects on growing-finishing pig performance. Feeding CLA to growing-finishing pigs improved average daily gain and gain / feed. This increase in average daily gain without an increase in average daily feed intake demonstrated by CLA would save millions of tons of feed per year in the growing-finishing phase for pigs in the United States. Feeding CLA reduced tenth rib fat depth for pigs fed .12, .25, and .50% CLA relative to controls. Decreased fat deposition will produce less waste from trim. Belly firmness was dramatically improved by CLA supplementation, this increased firmness may improve both bacon slicability and yield. CLA was deposited into both subcutaneous fat and lean tissue at significant rates that mirrored the percentage of CLA addition in the diet, this incorporation into lean tissue should positively impact the health of consumers. Further study to define the most appropriate dose requirement to optimize pig performance and body composition must take place. Also, identifying the active isomer(s) and method of biological activity must be defined.

Secondly, dietary supplementation of CLA maintained existing loin chop and fresh pork patty sensory and quality characteristics. Dietary supplementation of CLA did not change subjective color, firmness or marbling scores nor did CLA effect the percentage of total extracted lipids in loin tissue. CLA did not impact 24-hour pH, water holding capacity or Warner-Bratzler shear force of loin chops at day one postmortem. Day one postmortem loin chops were more red (Hunter a*) than were those from controls. The difference in Hunter a* however, was not maintained in loin chops held for 7, 14, 28 or 56 days of fresh

storage. This increased redness without other sensory or quality changes suggests that CLA may have positive effects on initial pork color. Panelists were unable to discern differences between chops from CLA fed pigs and controls at day one postmortem for sustained juiciness, tenderness, overall tenderness, or pork flavor. CLA at .50% extended pork color in fresh pork patties up to 7 days in fresh overwrap. Dietary supplementation of CLA in growing-finishing pigs will provide loins that are more red at day one postmortem, allowing them to potentially be selected for the higher value export market.

Finally, this study demonstrated that growing-finishing pigs fed CLA grew faster and more efficiently without having a compromised immune system or producing any pathological or disease state. Determination of the breed or breed cross that duplicates human atherosclerosis and other disease states most closely could provide excellent models for future research. Further study to determine the effects of long term feeding of CLA to pigs may also provide excellent information relating to atherosclerosis in humans.

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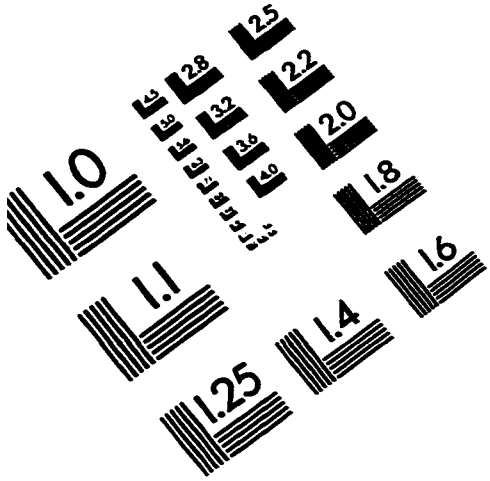
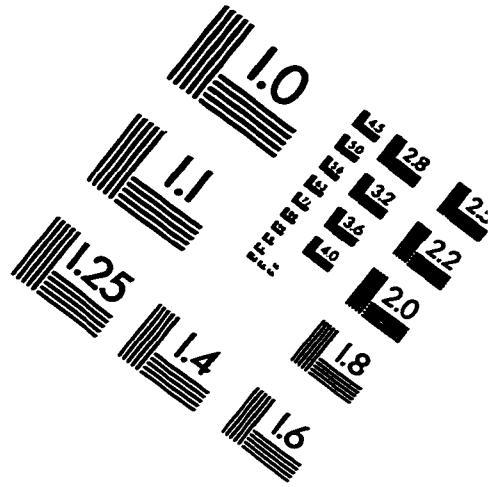
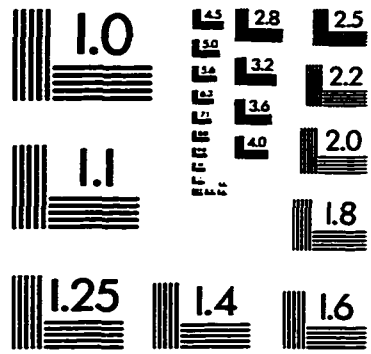
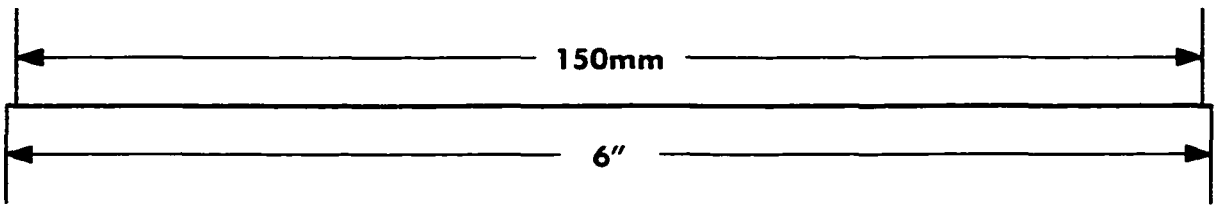
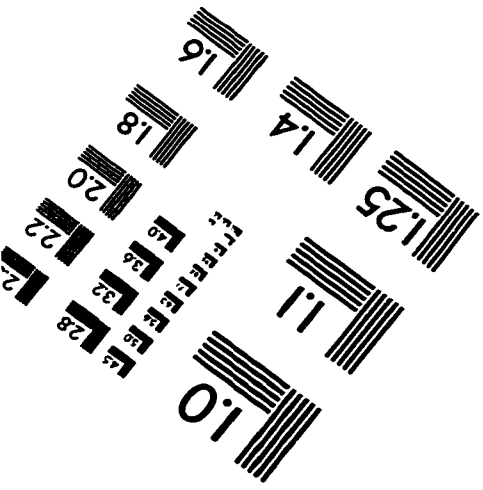
IMAGE EVALUATION
TEST TARGET (QA-3)

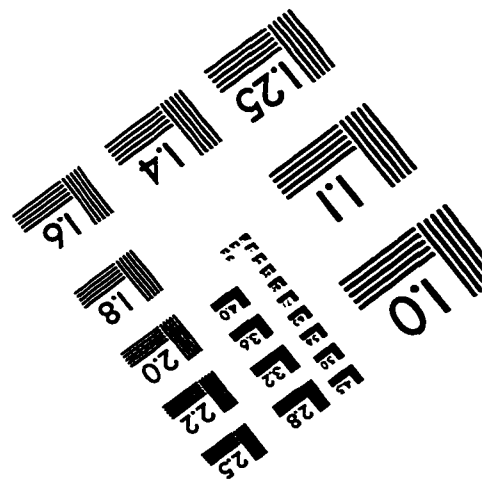












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